BIRLA CENTRAL LIBRARY
PILANI (Rajasthan)

Class No 590.7
Book No W46C
Accession No. ~~18612~~

The Collection *and* Preservation *of* Animal Forms

by

MORRIS MILLER WELLS, PH. D.

Copyright 1932 by

GENERAL BIOLOGICAL SUPPLY HOUSE
Incorporated
761-763 EAST SIXTY-NINTH PLACE
CHICAGO

PREFACE

THIS book contains information concerning the *Collection* and *Preservation* of those of our North American animals which are used in the Biological laboratories of our country. The author, Dr. Morris Miller Wells, intended that the material herein contained should compose the last chapter of his manuscript on "The Natural History of North American Animals with Special Reference to Laboratory Forms."

We wish to explain here, briefly, something of the purpose and present status of the "Natural History" just mentioned.

The Biology teachers of the country have had, up to the present time, no reference book which has included all or nearly all of the information which they have needed, about the animals studied in Zoology or Biology courses. Certain books contain descriptions of the details of the structure of the animals, in other volumes are discussions of the animals' economic importance, still other books list species, but no book of which we are aware embraces all of the different phases of animal life about which a teacher needs to be informed.

During the years when Dr. Wells was a teacher of Zoology at the University of Chicago and later as president of the General Biological Supply House, the need for a reference book covering these different phases of animal life was impressed upon him. In response to this need, he took upon himself the task of writing such a book. This work, as previously stated, is called "A Natural History of North American Animals, with Special Reference to Laboratory Forms." In the preparation of the manuscript for this Natural History, he spent most of his available time for ten years.

The general outline of the "Natural History" is as follows: At the beginning of each chapter a classification of the group under discussion is given. Next is a *Terminology,* in which terms used in describing the group are defined. Then follows a description of the *Structure, Habit, Reproduction, Life History, Common Species, Economic Importance* and *Collection* and *Preservation* of each member of the group which is commonly used in the laboratory and in certain cases of those animals of special scientific interest.

Before his death, Dr. Wells had completed the first copy of the manuscript and had begun the revision of the text. The completion of the revision of the manuscript is going on at present.

Because of the demand, however, for information on the Collection and Preservation of animals, it has been thought advisable to assemble and print this information as a separate book and to publish this book before the appearance in print of the other chapters of the Natural History.

In addition to the material gained from his own observations, Dr. Wells has included some of the methods for the preservation of certain marine forms, which are described in the bulletin of the Naples Zoological Station.

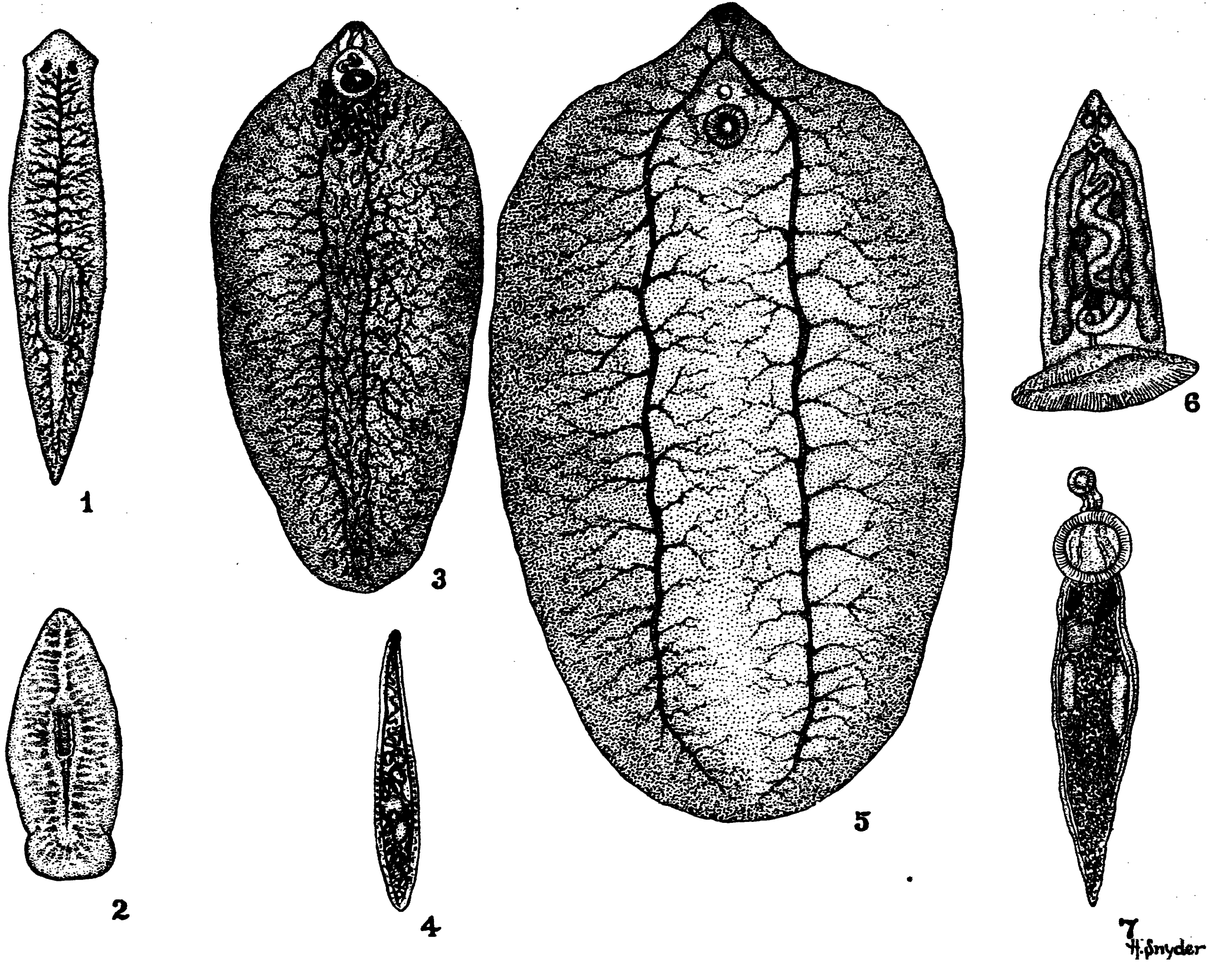
When it was decided to publish information on the "Collection and Preservation of Animals" as a separate book, every member of the staff of the General Biological Supply House was called upon to contribute what he thought might be of value in his particular field, as well as to read critically the whole manuscript.

We wish to express our thanks to these members of the staff for their efficient and whole-hearted cooperation.

EDITH BRADLEY WELLS.

May, 1932.

PLATYHELMINTHES



1. **Planaria.** Fresh-water Turbellarian.
2. **Bdelloura.** Limulus planarian.
3. **Fasciola hepatica.** Sheep liver fluke.
4. **Pneumoneces.** Frog lung fluke.
5. **Fascioloides magna.** Liver fluke of domestic animals.
6. **Diplodiscus.** From amphibian rectum.
7. **Gorgoderina.** Frog bladder worm.

(This illustration is typical of those prepared for "A Natural History of North American Animals with Special Reference to Laboratory Forms.")

COLLECTING AND PRESERVING ANIMALS

Introduction

WHILE it is not possible within the limits of the space and knowledge available to the writer to go into the details of securing and preparing for scientific use all of the many species of animals that are taken for this purpose, the principles for such work can be presented in outline. An attempt to do this is made in the following pages.

The first stage in the preparation of material for scientific use lies in the securing or collecting of the living specimens, for to do the best work one must always start with animals that are alive and in the best of health. The collecting of many specimens is not easy, however, while the capture of certain animal species may require the expenditure of a large amount of time, energy and money as is witnessed by the carefully organized expeditions that are sent out by our various museums and universities. If, however, one may attempt a list of rules for the beginning collector, they might be set down somewhat as follows:

1. The collector must be ready to work hard and long.
2. He must possess a fair share, at least, of mechanical skill and ingenuity.
3. Nothing must be allowed to interfere with the collecting work, for, to the collector, opportunity often knocks but once over long periods of years or even in a lifetime.
4. Specimens once collected must be attended to at once: much hard-earned material is ruined because the tired collector postpones caring for it until the next day.

The collector's training and equipment will vary widely with the species and quantity of material sought, and what is said in the following paragraphs has to do with such collecting as may be done by the individual, for no attempt can be made here to consider the organization of a large collecting expedition.

To the collector, animals may be grouped into three main divisions, since animals may, in general, be said to inhabit (1) the land, (2) fresh water, or (3) the ocean, and are to be sought in one or another of these three great earth regions. While it is true that the habits of the animals in any one of the three great habitat groups may vary widely, still the general methods of collecting are more or less similar for all and will be so considered here.

Beginning with the terrestrial animals, we find that terrestrial collecting has to do largely with the insects, amphibians, reptiles, birds and mammals, for while other groups such as annelid worms, crustaceans, etc., have their terrestrial representatives, still for the most part these minor groups do not occupy a great deal of the average collector's time. In fresh water we find protozoans, coelenterates, sponges, worms, crustaceans, molluscs, fish and amphibians in abundance, while certain reptiles, birds and mammals are also present. The great bulk of commercial collecting in the sea has to do with the crustacea, the molluscs, and the fishes, but protozoa, sponges, coelenterates, worms, and echinoderms are abundant. Marine amphibians are not known; the reptiles are present though few; birds are common, and the great size of the marine mammals makes up somewhat for what this group lacks in numbers in its marine population.

I Terrestrial Collecting

Collecting on land occupies the attention of trappers, gunners and big-game hunters, but the infinite details concerned in making a successful set for the wily mink, or in following the spoor of the none less cunning tiger or other big-game species must be sought for elsewhere than in these pages. In fact, to the teaching biologist, terrestrial collecting is confined largely to the methods of the entomologist who is confronted by the problem of devising means for securing specimens of the million or more species of insects that are existent on the earth. Many of the entomologist's troubles have to do with the making or securing of satisfactory nets for the snaring of his specimens. The ideal insect net should be capable of being folded so that it may be carried easily in one's pocket, yet it should be so strong and durable that one may with gusto beat grass, shrubs and trees without injury to the net. Unfortunately, since no such wonder net is available, the insect collector must make shift to do with those less ideal but more obtainable. The practical insect net, then, will have a collapsible hoop, a bag that is long enough to fold over the rim but which will, when desired, of itself hang loosely open to reveal clearly the catch at the bottom. Heavier nets are used to sweep insects from the vegetation, while inverted umbrellas or sheets may be spread beneath shrubs and trees to catch specimens that are shaken from their holdings when the tree is beaten with a club.

Time to Collect

A general habit displayed by many animal species, which plays into the collector's hand, is for the animals to gather in small or large groups at the breeding season. At this time also, many animals lose much of their natural timidity, and can, therefore, be taken in quantities at this season. The wise collector, however, will hesitate to destroy the source of supply by taking the breeding individuals, for it is such unthinking and often merciless activities upon the part of hunters, trappers and collectors in general that is to a considerable extent responsible for the disappearance or scarcity of many of our common species. The frog hunter who collects 500 big female frogs a day in the breeding season may be destroying, at the same time, 500,000 possible tadpoles and one-tenth this number of young summer frogs. Most of the insects may be excepted from this suggested protection since they are largely detrimental in any case. Further, the insects seem in little danger of extinction at the present time from any cause.

There are seasons other than the breeding period, however, when animals may congregate, as for instance in regions of abundant food or at the migration and hibernation seasons when they may be taken without injury to the following generations. Thus frogs congregate in low, moist fields to feed; monarch and other butterflies flock together for the southward migration in late summer; many reptiles and amphibia congregate in small to large groups at hibernation time, etc.

In any case, *it is highly essential that the collector know thoroughly the habits and life history of the form to be collected* if he is to succeed. I have many times been astonished to learn how much more some untutored trapper or hunter knew about the habits of certain conspicuous or commercially valuable animals than I did myself, although I may have read extensively concerning the species. As a matter of fact, there is available in every backwoods community a rich vein of knowledge concerning the natural history of our common animals, for these men who trap or hunt for a living come into the closest contact possible with the wild forms which they pursue. Frequently they have made observations of facts unknown to scientists, but unfortunately it is never safe to accept their statements without investigation, the real task being to separate the facts from superstition, folk tales and hearsay.

II Aquatic Collecting

Aquatic animals live in two general types of habitat, namely fresh water, including lakes, rivers, springs, ponds, etc., and salt water, which means the seas of the earth with a few inland lakes whose water contains a high percentage of salt. The animal population of the inland salt-water lakes, as for example our own Great Salt Lake, is not large, consisting as it largely does of certain highly specialized types of insects and insect larvae. Fresh-water fish cannot live in the salt water of the inland lake and the marine fish have little opportunity to try.

Fresh-water Collecting

The animals of fresh water include representatives of most of the animal phyla with the worms, arthropods, molluscs and fish the most conspicuous inhabitants. There are no fresh-water echinoderms, but this lack is offset by the fact that the amphibia do not live in salt water. The reptiles and mammals and to some extent the birds are also common fresh-water inhabitants.

As far as the biologist is concerned, this type of collecting is usually done either by hand or with the aid of hand nets, dip nets, and small seines. The collecting equipment required for most biological work in fresh water, however, is simple when compared with that used in marine collecting.

Marine Collecting

The methods and materials required for extensive marine investigation and collecting differ greatly both in type and quantity from those used in fresh water. The fresh-water biologist works for the most part with light equipment in or on small, usually quiet, bodies of water. To one who has done his initial collecting in fresh water, the ocean at first presents an almost fearsome aspect with its never-tiring waves constantly roaring shoreward but shifting mysteriously with the changing tides, and it is the irresistible forces locked in the ocean that account for the special methods and equipment that must be used in marine collecting. For one thing, the tides regulate largely the time of collecting, since little can be done on any coast except at low tide. Again, the constantly moving water with the waves, currents, tidal rips, etc., warn the collector to be ever on the alert, since catastrophe will almost surely follow the slightest carelessness or negligence. Again, many of the desired marine forms live in comparatively deep water, thus making necessary large boats and heavy tackle for their taking. Finally, many if not most marine invertebrates change their shape at the slightest unusual stimulus which means that such species must be prepared according to special methods if they are to be preserved in the active, expanded state.

The oceanic animals of most interest to the general biologist are the protozoa, sponges, coelenterates, worms, arthropods, molluscs and echinoderms. Fish are abundant in the sea. Amphibia are not present. Reptiles are rare, as are also the mammals. While certain birds occur in great numbers in salt or brackish water, the birds are after all a terrestrial group, all of the water birds coming ashore at least during the breeding season.

Plankton. One of the most interesting ecological or habit groups found in aquatic environment and especially in oceanic waters is made up of those animals, and plants also, that float about with the water currents. Oceanic plankton offers a myriad of constantly changing forms for the biologist's attention and may at times almost pack the water with life. Plankton is taken by drawing through the water behind a boat, a fine-meshed net made of some strong cloth (e.g., silk bolting cloth), the net straining the animals from the water. The fine-meshed plankton nets will capture even the smaller of the microscopic forms. It should be pointed out, however, that there are large quantities of plankton consisting of unicellular organisms such as Chrysomonads and Rhodomonas which are so small that the finest nets do not capture them. This is the so-called nanno—or centrifuge plankton of Lohmann (1911), and it is thought that this plankton, which can be secured only by centrifuging water that has already been strained through the finest mesh net, may be fully as important a food factor in the diet of those forms that regularly live on plankton as are the still minute but larger forms such as the diatoms and dinoflagellates.

The plankton taken by towing will vary with season, latitude, locality and the type of net. Such forms as the *Radiolaria, Foraminifera* and *Dinoflagellata,* will represent the protozoa in great numbers. Most of the invertebrate sea animals pass through a free-living, floating stage during the life cycle as do also the lower chordates and many of the fishes. Thus, plankton may contain the amphiblastula of sponges, the tiny medusae of the hydrozoa, the bipinnaria and brachiolaria of echinoderms, the tornaria of Balanoglossus, pilidia, and trochophore larvae of worms, the veliger larvae of the molluscs, the zoea of crabs, the schizopods of the lobster, pelagic fish eggs, etc., etc. Among the smaller adult plankton forms the *Entomostraca* such as copepods and cladocerans sometimes swarm in such countless numbers as to color the waters of the sea over large areas. The larger plankton species include the larger jellyfish; the *Syphonophora, Physalia* and *Velella;* the *Ctenophora* such as Beroe, and many other coelenterates, the intensive population of the open sea ever offering new and curious forms for the attention of the marine investigator.

To outline briefly the method of preparing many if not most of the invertebrate marine forms, which in fact is to say most marine animals in which the average collector is interested, we may offer the following steps:

1. Collect the animals, keeping them in clear uncontaminated sea water.
2. Transfer to deck of boat, or if possible to shore laboratory.
3. Allow the specimens to expand either in clusters or singly as seems best.
4. Anesthetize carefully in the expanded condition.
5. Pour or siphon off as much water as is possible without causing the animals to contract.
6. Pour over the specimen or specimens the proper killing and fixing solution. Sometimes the fixative should be poured on with a rush, sometimes it should be added slowly.
7. After the proper time in the killing solution pour off the fluid or transfer the animal to the proper grade alcohol. (Grade specified for various forms on the following pages.)
8. Run up to 70% alcohol through changes of 35% and 50%.
9. Change the alcohol daily until it no longer becomes discolored.
10. Store in 70% alcohol, being sure to use a sufficient volume of fluid per volume of specimens, say 5 or 10 volumes of alcohol to one, depending upon the nature of the specimen.

The above ten points should, if carefully followed through with the necessary specific variations, produce satisfactory museum material. For general class work it is not usually required that the preparateur work with so much detail. The preparation of class specimens is outlined for many of the common laboratory species in the following pages.

Directions for Collecting and Preserving Animals

For the most part, the directions given here for killing and preserving animal specimens must be general, since only in certain groups, where some particular species is important above all others, can specific directions be given. The experienced preparateur need not be told, but the beginner must ever bear in mind the fact that even closely related species and sometimes individuals of the same species may react quite differently to the anesthetic or to the killing and fixing reagents so that some experimental work will likely be necessary for each species. With the aid of the general methods outlined in the following pages, however, it should not be difficult to ascertain, after a few trials, which solution and methods are most satisfactory in the preparation of any given form. A list of the formulae for common preservatives, anaesthetics and killing fluids is included in the appendix of this book.

We cannot discuss in this booklet the various methods of mummifying, infiltrating with paraffin, casting in plaster, celluloid, etc., which are so commonly used in museums for the perfecting of their habitat groups. The Turtox Service Department will be glad to furnish sources of dependable information on these subjects.

PROTOZOA

The Collection of Protozoa

PROTOZOA may either be collected in the open or reared in the laboratory. Terrestrial protozoa are common in soil and semi-fluid environments, but can scarcely be secured in quantity except by the use of culture methods. Many of the fresh-water protozoa can be grown in the laboratory, while at times one may run across natural cultures in the field that are almost pure for one to a few species. For example, Euglena may be skimmed from the surface of the water of shallow stagnant ponds in summer. Ciliates will be found in abundance in stagnant water which contains a large amount of decomposing organic matter with an abundance of bacteria. Rhizopods in general require a much fresher environment, being usually plentiful in the brownish, richly diatomaceous scum on the surfaces of dead leaves in shallow pools, and on the stems of water plants. Sporozoa are parasitic and each must be sought for in its specific host.

Ameba: Culture Methods

Ameba may be secured in small numbers, in scrapings from the stalk and leaf surfaces of pond plants. To secure the animals in quantity, it is best that they be grown in cultures. This may be done fairly easily as follows: boil a handful of fresh green water-weeds (*Elodea* or *Ceratophyllum*) for fifteen minutes, or long enough to kill them. Place these boiled weeds in a jar or jars of boiled pond or distilled water, putting only enough weeds in each jar to cover the bottom loosely. The quantity of weed material used is important and a little testing will be necessary before the right amount is found. Usually, the mistake of putting in too much weed material is made at first. Add to the jar a few grains of thoroughly boiled wheat. Fill the jar one to two inches deep with clear pond water which has been boiled, and cover with a glass plate. Allow the culture to stand for a few days and inoculate with amebae, either from another culture or from fresh pond scrapings. If the culture proves successful, the water will remain clear and a loose mat of greenish material will form at the bottom. Microscopic examination will show that this mat is made up of diatoms and desmids, as well as other single-celled plants. The amebae will be found at the bottom and sides of the jar and even at the surface of the water. Amebae may be made continuously available by preparing a new set of cultures every three or four weeks, since this is about the length of time a single culture may be expected to last. The growing of specially large amebae does not depend so much upon the favorableness of the conditions, as it does upon the size of the species with which the culture is inoculated. The presence of large amebae in a culture indicates the presence of a large species, and if a new culture be inoculated with such large species, large amebae will be available.

The common soil ameba, *Ameba limax*, may usually be found in abundance in mixed cultures in which ciliates flourish.

Formanifera and **Radiolaria** may be obtained by carefully washing sand or mud collected on the beach at different levels between tide-marks. They may also be washed from dredgings or scraped from the surface or washed from the roots of sea weeds. Sorting of Formanifera shells is done by using fine silk bolting cloth or gauze screens.

Paramecium: Culture Methods

Paramecium is so common and so abundant that one will usually have little difficulty in locating it. With the help of a hand lens, examination of a drop here and there taken from the surface film of pools, tanks, etc., will be certain to reveal the presence of paramecia without too much search. Water is, of course, necessary to the presence of living active paramecia and in dry regions, therefore, the search may be more or less limited. Water that contains paramecia in large numbers will usually also contain quantities of bacteria upon which the paramecia are feeding. Look for and examine all jars, barrels, or pools which possess a light scum over the surface.

Cultures of paramecia may be reared in great abundance with little equipment or trouble. To secure a pure and fairly permanent culture, clean a one-gallon jar thoroughly and fill it two-thirds full of clear uncontaminated water. To the water add thirty to fifty grains of wheat that have been thoroughly boiled. A few crumbs of white bread may also be added. Set the jar away in a part of the laboratory where the light is of medium intensity and within two or three days a scum, bacterial in origin, will have appeared on the surface of the water in the jar. When this scum has appeared, inoculate the culture by adding a pipette full of paramecia from some source such as a mixed culture of a hay infusion or a pool known to contain paramecia. No mistake need be made since the paramecia are easily visible to the naked eye in a pipette. Place a plate over the jar and let it stand for a few days more. By this time the successful culture will contain enormous numbers of paramecia. The animals will occur near the surface at first, but later streamers of closely packed animals may extend from the surface film down into the lower water until the entire jar gives the appearance of being literally alive with paramecia. Paramecia may be killed for examination by dropping them into a saturated solution of mercuric chloride or into Bouin's fluid.

Euglena: Culture Methods

Euglena, as compared with paramecium, requires a culture to which considerably more starchy food material has been added. Boil a few grains of rice—then pulverize and place in a jar of water (6x8-inch battery jar) and cover with a glass plate. Let the jar stand for a day or two and then inoculate with euglena which have been collected as described above. Euglena cultures must be placed where they will be exposed to the sun for at least part of the day. They can stand direct sunlight of rather high intensity without damage. By the addition of water to the cultures from time to time, to replace that lost by evaporation, a euglena culture may be kept going for months. In the euglena culture, as in the open pond, one usually finds encysted and motile stages appearing side by side.

To preserve in extended condition, these animals should be allowed to expand in sunlight in a minimum quantity of water and then killed by dashing boiling hot killing fluid (Bouin's) over them.

The Preservation of Protozoa

The preservation of protozoa is usually not a matter for the consideration of the preparateur of laboratory material since in the laboratory most protozoa are studied as live material or mounted slides. It is surprising at first to find that soft bodied forms such as ameba and paramecium require killing in the strongest killing fluids. One reason for this is that the animals must be killed so quickly that they will not have time to change their shape in reacting to the stimulus of the killing fluid. This is especially true of the Rhizopoda which tend to draw in their pseudopods quickly if stimulated. Bichloride of mercury in saturated solution will successfully kill many of the protozoa. Hot Bouin's fluid is also satisfactory for certain types, e.g., *Euglena*. For further details as to this technique, one can but refer the reader to the various books on microscopic technique, although so far as the author knows there is at present no satisfactory discussion of this subject available in any of the literature.

Radiolaria may be placed directly in fifty per cent alcohol, and after a few hours transferred to seventy per cent. Good preparations may also be made by dropping a few drops of one per cent osmic acid into the sea water containing the animals and then transferring them to alcohol after washing in fresh water. Excellent microscopic preparations of some of these species of minute pelagic organisms have been made by a treatment with a saturated solution of corrosive sublimate or with iodized 35% alcohol.

Opalina may be secured in quantity from the intestines of frogs and toads, especially the latter, in whose large intestines they may be present in enormous numbers. The intestine of a freshly killed frog or toad may be slit and the contents washed into a small amount of normal salt solution. The protozoa can then be studied under the microscope. To make permanent mounts, kill in Bouin's or Gilson's fluid, wash and stain in acid carmine or cochineal: destain to bring out the nuclei and run into thin balsam for mounting.

Gregarines. The intestine of the ordinary mealworm (*Tenebrio* larva) usually contains gregarines in large numbers. Clip off the head of the worm and, with forceps, pull the two posterior segments from the body. The intestine will come out with them. It should be opened in salt solution and examined under a binocular microscope. If gregarines are found they may be fixed in Bouin's fluid, stained and mounted.

The *Vorticellidae* are best killed with boiling saturated corrosive sublimate solution, run up through three changes of alcohols to seventy per cent alcohol and stored in seventy per cent alcohol.

PORIFERA

Sponges

The Collection of Sponges

FRESH-WATER sponges are often abundant in clear rivers, sloughs and lakes in mid-summer and may be secured in considerable quantity at this time. The collecting will consist of cutting off the stems of reeds or other aquatic vegetation upon which the sponge may be growing, or of carefully lifting the growth from its location on submerged timbers or stones. A flat-bladed knife is the best instrument for this purpose. *Spongilla* is often plentiful on pipes at the outlet of reservoirs. If specimens bearing winter bodies are wanted they should be collected rather late in the season.

Marine sponges are secured by commercial spongers with the aid of sponging hooks, diving outfits, etc. The commercial sponge of the sea, like its fresh-water relative, will always be found growing attached to some firm support such as a coral rock and must be torn from this support in the act of collecting. If care be taken not to tear the sponge, nothing should prevent a successful end to the beginning collector's first efforts. *Grantia* will be found growing upon the submerged portions of old docks at about the low-tide water-mark.*

The Preservation of Sponges

The mistake most frequently made in preserving sponges is to place them in formalin solution rather than in alcohol. All horny sponges and probably all types of sponges are best preserved in alcohol, for in formalin the sponge tissue disintegrates until only the skeleton is left and eventually this may break down also. It is likely that the acidity of the formalin is the detrimental factor in this solution and experiments to determine whether or not this is the case need to be carried out. In alcohol practically all sponges preserve indefinitely in good condition. The horny sponges may be dried with the flesh on to good advantage. The skeleton is the part of the sponge with which we are best acquainted and requires no preservation once it is cleaned.

For sponges which are to be used for exhibition, it is enough to immerse them directly in 70 per cent alcohol, renewing it when it becomes discolored. To avoid contraction in some species, the animals should be fixed in chromic acid of 1 per cent solution for about half an hour, or in saturated corrosive sublimate for fifteen minutes. Those sponges which are to serve for study, if they are not too large—that is, if they are not more than 10 cm. (4 inches) in diameter—are best immersed in 90 per cent alcohol or in absolute alcohol, which should be renewed after three or four hours, and again after twenty-four to forty-eight hours. If the specimens are too large, small pieces may be cut off and treated in this manner.

If they are to be dried, they should be washed first in fresh water for a few hours, then should lie for about a day in ordinary alcohol, and then be placed in the air or in the sun. If treated in this way, sponges will not have an offensive odor. If it is desired to retain the rosy color of certain sponges (*Suberites, Axinella*) for several days, it is enough to place them in 40 per cent alcohol and not change it.

---

*A detailed description of the commercial collection of sponges is given in the chapter on *Porifera* in the "Natural History of North American Animals," by Morris Miller Wells.

COELENTERATA

The Collection of Coelenterates

HYDRA is the only fresh-water coelenterate that is available to the average collector. Hydra of one or more of the three best-known species will usually be found inhabiting the clear waters of lagoons, ponds, rivers, etc., in much the same environment that supports the fresh-water sponge. Hydra is a sessile animal for the most part, spending its time suspended from, or at least clinging to, the vegetation, stones, timbers, fallen leaves or in fact to the surface of any object in the water. The animals may be removed from the stones and other heavy bodies by lifting them away carefully with a thin-edged scalpel or by washing them off into the collecting pan with a stream of water directed at them from a large rubber bulb pipette.

A good method for bringing hydra into the laboratory is to locate an area of vegetation such as Elodea or Chara upon which the animals are growing profusely. That the hydra are present in numbers can be determined by examining samples of the vegetation in water in a pint or quart glass jar held up to the light, when the hydras can readily be seen on the leaves and stems of the plants. Once located, the vegetation can be collected in pails, without water, and taken to the laboratory. If it is desired to start permanent cultures, the vegetation should be scattered through the aquaria. If, however, a large supply of hydra is needed for immediate use, place the vegetation in wide pans, such as dish pans, filling the pans one-half to two-thirds full of the pond weeds and covering all with water until the pan is almost overflowing. By the next day, the hydra will have ascended from the vegetation to cling to the surface film where they may be secured with a pipette or even skimmed off in large numbers.

Marine Coelenterata vary widely in habit and collecting methods must consequently vary as widely. Further, in collecting marine coelenterates the collector, in most cases, has to deal with both the sessile hydroid and the motile medusa stage. The result is that for laboratory purposes certain conspicuous and common forms have come to be used, while many hydroids and medusae are known in but the one stage; at least the relationship between hydroid and medusa is not yet clear in many cases.

The sessile hydroid stages of all of the groups of coelenterates are readily secured. Our eastern *Obelia* for example may be secured by cutting off the kelp leaves on which it grows, while the larger, more bushy Pacific coast forms can be detached in clusters from stones, leaves and pilings. Sea anemones of certain species, e.g., *Metridium marginatum*, can readily be removed from their rocky attachments and transferred to the laboratory in buckets. Sea-plumes, and corals of certain species grow in water only a few feet deep and can, at low tide, be broken away and transferred in tubs or pails of water to the firmer working ground of the laboratory table.

The motile medusa stages of coelenterates are, in the case of such tiny forms as the *Obelia* medusae, taken in plankton towings, or if the hydroid stages with gonangia present are placed in finger bowls of sea water, the medusae may emerge and swarm in the dish. The larger medusae of *Aurelia* occur in loose schools in the quieter waters of the bays during the summer months and may be dipped up and carefully poured into a waiting tub or bucket which rests alongside the collector in the bottom of his boat. Great pains must be taken in the handling of all of the medium to large jellyfish or important portions of the delicate structure will be lost by breakage. In taking the larger forms such as *Cyanea,* there should be mentioned the further danger of the collector's receiving painful burns from contact with the nematocyst-laden tentacles which will frequently be whipped about in the most unexpected fashion by the veering breeze.

The Preservation of Coelenterates

The coelenterates consist of a widely diversified group in so far as laboratory preparation is concerned and so many different techniques are used in the preparation of specimens from this group that only the more generally used methods may be outlined here. The life history in the coelenterates, besides showing an alternation of an asexual with a sexual generation, may also contain several different stages in each generation.

HYDROZOA

In the *Hydrozoa* the hydroid stages must be preserved with extended zooids if a detailed study is to be made. To do this the hydroids must be collected and taken to the laboratory, there to be treated with a solution of some anesthetic. The addition of magnesium sulphate (Epsom salts) in large quantities to the sea water in which the hydroids are confined will usually result in complete anesthesia within an hour to a few hours in the case of many hydroid species. Carefully pipette off the excess sea water. Test the progress of anesthesia by touching the tentacles with a bristle. Ten to fifteen minutes *after* the hydroids fail to respond to this stimulus add gradually four per cent sea-water formalin to kill. A saturated solution of chloretone may in some cases give better results than the magnesium sulphate, or if available, cocaine is a valuable anesthetic for this purpose. The medusae of the hydrozoa may be preserved in neutral formalin, the strength of the solution being determined for the species in question by test experiments.

Hydra—The problem in the preservation of hydra is to kill the animals in extended condition and details of method must be worked out for the material at hand. In general, one first collects the hydra, being careful not to injure them, and distributes them in petri dishes or shallow glass bowls containing but a film of water. The hydra will, if in good condition, extend in the water film and may then be killed by quickly flooding them with an excess of boiling hot Gilson's or Bouin's killing fluid. Several dozen specimens may be killed at one time in this manner. Should the hydra expand incompletely in the film, poor conditions upon the part of the animals is indicated and a new supply should be obtained. Further, the specimens should be killed at the first expansion, for if left overly long they will become torpid and will lie quiescent in a half-expanded or fully-contracted condition until death occurs. Better results will be obtained if time permits the killing of the specimens singly, and if they are "shot" with pipette from base to peristome. This extends the tentacles.

All the *Hydroidea*—that is to say, the permanently polypoid forms, with very rare exceptions—are best killed with hot saturated sublimate. If the polyps are not in complete expansion when received, the colonies should be allowed to expand in beakers of fresh sea water. As soon as the fixing fluid has been poured over the animals, the whole should be turned into a crystallizing dish containing fresh water, to cool. Then the animals should be removed to another dish of fresh water for washing and after five minutes to weak alcohol (50 per cent). If it is desired to avoid the heating of the liquid, cold sublimate can be used, but only for the Tubularidae.

Large colonies of *Tubularia* and *Pennaria* may be killed with the mixture of sublimate and chromic acid in volume equal to that of the water in which they are being kept, and after a few minutes washed and removed to alcohol.

Certain medusae of the *Tubularidae* are killed with the corrosive sublimate-acetic solution used in large proportion, or with Kleinenberg's solution. Others may be killed with concentrated acetic acid and immediately poured into a tube containing a mixture of alcohol and chromic acid. If the tube then be agitated gently the animals will regain their normal form. After remaining in the mixture about fifteen minutes place them in 35 per cent alcohol and then gradually transfer to that of 70 per cent. Another and perhaps better way of handling these forms is to allow them to expand in a specimen tube less than half full of water and when they are well extended fill the tube with acetic acid. Then transfer at once into the mixture of alcohol and chromic acid. A few minutes later pour out a small portion of the liquid in the tube and add chromic acid, because considerable water will have gone over with the animals. After fifteen minutes wash in fresh water and transfer to 35 per cent alcohol. Still another method is to use the chrom-osmic acid mixture as a hardening medium, but the animals do not remain as transparent and the tentacles are somewhat contracted.

During the hardening process, especially when many of the medusae are treated at the same time, the tube should be held in a horizontal position in such a way that the bells are down on the side of the tube and the animals not in contact with each other. For the final preservation of certain forms place them in alcohol in a small tube, separating the individuals by wads of cotton, and put this into the exhibition jar.

Medusae of the *Campanularidae*, e.g. *Obelia*, are fixed in the mixture of sulphate of copper and sublimate and after a few minutes washed in fresh water until every trace of precipitate has vanished, and then placed in weak alcohol, and so on, up to 70%. Some collectors report that obelia can be expanded by dropping formaldehyde slowly into the sea water in which they are immersed.

The *Trachomedusae* should be fixed in the chrom-osmic liquid for five to twenty minutes, according to their size, washed in fresh water, and gradually transferred to alcohol. *Cunina* is better when killed with concentrated acetic acid before being hardened with the chrom-osmic mixture. A simpler method is to use formalin of 4 per cent and chromic acid of 1 per cent in equal parts for killing and hardening. Allow the specimens to remain in this mixture from one to two hours; then wash in fresh water and transfer to alcohol.

To prevent the flattening of the bell of large forms place a watch glass in the bottom of the jar and rest the bell of the hydromedusa in its concave side.

Siphonophora—As with the *Hydromedusae*, the preparation of the *Siphonophora* should be accomplished as soon as possible after capture, and only those specimens should be treated which are in good living condition. The siphonophores will disintegrate if kept at laboratory temperature for no longer than a few hours, though frequently the breaking up may not take place until the colony is brought into contact with the fixing fluid. Much care must also be exercised not to shake roughly the vessel which contains the animals before they have been killed. It has often been observed that a trace of an acid or other reagent in the water is enough to destroy the colony. The receiving vessel must be perfectly clean.

Velella is killed with the chrom-picric mixture, or with that of sublimate and chromic acid, and after a few minutes removed to weak alcohol.

Porpita is slowly killed by dropping with a pipette a few drops of Kleinenberg's solution into the beaker where it has become distended. When the beautiful blue color of the colony has begun to turn red as an effect of the acid, it should be removed to the Kleinenberg solution, where it may remain fifteen minutes before it is put into weak alcohol.

Physalia—The Portugese Man-o'-War is frequently preserved in formalin without attempt at asphyxiation. This method results in an enormous shortening of the tentacles and some distortion of the air sac or pneumatophore, but nevertheless results in usable specimens. In handling this material one must learn to avoid contact with the exceedingly "hot" tentacles which are readily blown about when the animal is lifted out of the water by the sail, or pneumatophore. A method for preparing museum specimens is to allow each individual Man-o'-War to expand its appendages and polyps in a tall cylinder filled with clear sea water. The preparation succeeds much better in a very high cylinder on account of the great extensibility of the nettle-filaments. When the colony is well distended pour over it a volume of the sublimate and acetic acid mixture equal to one-fourth that of the sea water containing it. As soon as dead, the colony should be transferred to a similar cylinder containing chromic acid of one-half of 1 per cent and after twenty minutes to 50 per cent alcohol and finally to 70 per cent.

SCYPHOZOA

The *Scyphozoa* offer some interesting problems to the preparateur, most of which have to do with securing satisfactory preservations with the animals extended. Asphyxiation by magnesium sulphate or cocaine is commonly used with the Atlantic anemone, *Metridium*. This method, however, gives but mediocre results with many of the forms of this group.

Charybdaea should be killed with the chrom-acetic mixture No. 2 and immediately afterwards treated with chromic acid one-half of 1 per cent. After a half hour transfer to alcohol, taking care for the proper suspension of the tentacles.

A common method for preparing the Atlantic coast *Aurelia aurita* for class work is to dip the animals from the water, placing them directly in pails or tubs with plenty of sea-water formalin. In the laboratory the animals are lifted carefully one at a time and placed in a weak concentration of neutral formalin. A 2% solution should be about right, although this concentration will vary with the region in which the medusae are taken. A little experimentation will discover a concentration that will neither shrink the animals nor allow them to disintegrate. If the specimens are to be kept for some time they should be transferred to a fresh solution after a few days. Further, if the animals are to be transported or shipped they must be packed not too tightly in jars or cans that are filled to the top with the neutral formalin solution. If even a small bubble of air is left in the container it will produce a sufficient disturbance as the containers are handled by the express company or post office to break up all of the specimens more or less completely.

The ephyra of such forms as *Pelagia, Rhizostoma* and *Aurelia* may be killed by pouring into the sea water containing them, 3 parts of a 1 per cent solution of osmic acid. When the ephyra have just begun to take on a brown tint they should be washed in fresh water and placed in 35 per cent alcohol, then into 50 per cent and finally into 70 per cent. Formalin of 4 per cent may be used with excellent results with these animals, because it does not give the brown tint which is imparted by osmic acid. To avoid the flattening of the umbrella of Rhizostoma, the animal may be killed in an exhibition jar with a somewhat narrow neck. After the transfer to weak alcohol the mouth of the jar should be covered with a piece of bladder and should stand upside down, with the convex part of the bell resting in the neck. The medusa should remain in this position until the alcohol has been changed to 70 per cent and the whole body has become permeated with the fluid. When formalin is used for killing and hardening, this inverted position should be maintained from the first.

The larval forms, scyphistoma and strobila, may be killed with hot saturated sublimate. Strobila are also well fixed with a mixture consisting of 9 parts of concentrated acetic acid and 1 part of 1% osmic acid. From this they are quickly transferred to fresh water for washing and put into alcohol.

Pelagia noctiluca should remain in the chrom-osmic solution about an hour, and then be washed and placed in weak alcohol. In the alcohol the animal should be suspended by threads tied carefully to the extremity of each tentacle. See that the bell does not touch the bottom of the jar and let the animal remain thus until completely hardened.

Cassiopea—Formalin of from 2 to 3 per cent may be used to good advantage for killing and hardening this species. Another method is to treat with osmic acid, as was done with Rhizostoma, but when the brown tint begins to appear, a 5 per cent solution of bichromate of potassium should be substituted for the osmic acid and should be renewed after a few days. The animal ought to remain in this reagent for about two weeks, but it cannot remain much longer than that without suffering injury. Then remove the object to 35 per cent alcohol, then upgrade to 70 per cent. Numerous crystals of a salt are formed on the outside of the animal and a heavy precipitate falls to the bottom of the receptacle, making it necessary to change the alcohol frequently. A few drops of concentrated sulphuric acid should be added to the alcohol.

ANTHOZOA

The first thing to be done when an anthozoan has been caught is to place it in a receptacle with fresh sea water. It always happens that these animals, when disturbed by the fishing apparatus or transportation, contract or withdraw into themselves completely. To cause them to expand, it is enough to let them remain in a jar with pure sea water, although it may be necessary to keep them for a longer or shorter time in running water. When fully expanded they then may be suddenly immersed in the chrom-acetic mixture No. 2.

Since all the *Alcyonaria* contain minute calcareous spicules which furnish the specific characters, they should remain in an acid mixture as short a time as possible so that the acid may not attack the spicules. In those cases in which chrom-acetic mixture No. 2 has not given good results, a mixture of sublimate and acetic acid may be employed, but always for the killing alone. The animals should be transferred quickly to weak alcohol. A method used by G. von Koch is quickly to immerse the distended animals in absolute alcohol or 90% alcohol, making an injection of the same afterwards into the interior of the animals.

Usually when the colonies have become expanded, the water in the receptacle should be siphoned off until only enough remains to cover them. This operation needs to be performed with great care to avoid any shock which might cause retraction of the tentacles. Then pour rapidly into the jar a volume of chrom-acetic solution No. 2 double the amount of the water in which the animals remain, and immediately afterwards transfer them to 35 to 50 per cent alcohol, giving the preparation a few gentle shakes to free the tentacles and arrange them in natural position. Another good method of killing is with hot saturated sublimate, using the same proportions as of the chrom-acetic mixture, and washing the animals when scarcely dead in fresh water before the transfer to weak alcohol.

Gorgonia should be killed with chrom-acetic mixture No. 2 in the same dish in which they have become distended, on account of the great sensitiveness of their polyps. It is always advisable to have as little water as possible in the dish at the time of killing these animals, and to pour over them a volume of the mixture twice as great as that of the water in which they are. It has been noted that the *Gorgonidae* which have expanded in sea water which has begun to turn bad are those which have given the better preparations. The small colonies, or pieces of colonies, remain with their polyps distended, if they are killed with boiling saturated sublimate.

The sea-pens, *Stylatula*, when they have become well distended, may be taken by the naked base and very swiftly immersed in a tall, cylindrical jar containing the chrom-acetic mixture No. 2, and after a few seconds placed in a crystallizing dish containing 50 per cent alcohol, where they should be allowed to rest on their backs. Then with a small syringe with a very fine point inject alcohol into them through a minute hole made in the extremity of the base. In this manner the alcohol penetrates to all parts of the interior and distends the polyps. In some species the alcohol injection is unnecessary. Following the injection tie a thread around the end of the base above the hole which was made so that the escape of the alcohol may be prevented. After some hours the animals should be transferred to 70 per cent alcohol.

Corallium rubrum, after it has been allowed to expand in running sea water, should be killed with boiling saturated sublimate solution (half as much as the water containing the coral), and quickly transferred to weak alcohol. By this method the color is almost perfectly preserved, while by the use of the chrom-acetic mixture it is very much injured. The alcohol which has been used for this coral cannot well be used afterwards for the preparation of any delicate organism, because of the fact that it now stains objects red.

Zoantharia—All the species of *Antipathes* are fixed with saturated sublimate, which on account of the slight contractility of the polyps, always succeeds. The saturated sublimate, which is used cold, should be of the same volume as the water containing the animals.

Actiniaria (*Sea Anemones*)—The preparation of this group is very difficult, the great contractility and resistance of the muscular system of the majority of the species frequently constituting insurmountable obstacles to success. Many times when it is thought that the animal has been deprived of any sensitiveness, immersion in a reagent of rapid action is sufficient to show sudden and surprising contraction of the tentacles and of the whole body. When several specimens of certain forms are treated by the same method and under the same conditions, some die distended and the rest contracted. In some cases good results depend on circumstances which, up to the present time, are wholly unknown. After all, however, there are many species with which perfect results can be attained, if great care be exercised in the manipulations. After being well extended in running water, the animals are killed with the chrom-picric solution, used in volume equal to that of the water in which they are. This should be rapidly poured into the jar containing the actinian, after as much of the water therein has been poured off as may be and leave the animal immersed. Instead of plain chrom-picric, the following solution is now much used:

Chromic acid, of 1 per cent	1 part
Saturated solution picric acid	1 part
Formalin, of 4 per cent	1 part

When the animals die, they will fall from the sides of the glass and they should then be transferred to another jar containing one-half of one per cent chromic acid, where they should be suspended upside down by means of a glass float, the hook of which has been passed through the lower rim of the body. The animals should be gently shaken to give the tentacles a natural position. After half an hour they are placed in weak alcohol and then gradually transferred to 70 per cent solution. It is a good plan to suspend the animals upside down by means of a float in the final receptacle, though it is hardly worth while to do this for the smaller specimens.

Sagartia may be killed with boiling saturated sublimate and before they are transferred to alcohol, the animals should be allowed to harden for some minutes in ½ per cent chromic acid solution.

Edwardsia is slowly narcotized by dropping from time to time a few drops of 70 per cent alcohol into the sea water in which it is. It is then killed with hot saturated sublimate. Success depends upon the complete loss of sensitiveness, which may be tested by touching the tentacles with a needle point.

Larvae of the *Actinia* are killed with saturated sublimate or with chrom-acetic No. 2.

Coral colonies may be pried or broken from their attachment and allowed to die in fresh or tepid water, when the animal matter can be removed and the skeleton or corallum can be cleaned and bleached. To secure specimens of coral showing expanded polyps it is first necessary to collect colonies by breaking fragments from the coral reefs. Place these pieces in a pail or tub of sea water and set the whole in dim shade. Then with a pipette squirt a few drops of beef juice, or with forceps lower a small bit of crabmeat, onto one or more of the more prominent polyps. The feeding reaction will probably take place and soon the polyps all over the corallum will expand, when the animals may be killed with a mixture of boiling sublimate acetic as per the following directions for Madreporaria.

The *Madreporaria* may also be allowed to remain overnight in a beaker filled with clear sea water. The following morning usually shows the polyps fully expanded. Then, after pouring off a portion of the water (enough to leave the animals barely covered), kill them with a solution of boiling sublimate and acetic acid in volume equal to that of the sea water. Immediately afterwards transfer the colony to 35 per cent alcohol, making an injection of the alcohol into each polyp to keep it well distended. At each change of alcohol up to 70 per cent make a similar injection, and be sure to test the final solution with tincture of iodine to see that the sublimate has been eliminated.

CTENOPHORA

Ctenophora must be collected by dipping them from the water, as they are so fragile that they will tear apart if handled with a net. Preservation of the delicate forms such as the jellyfish and ctenophores is always difficult, many species going to pieces in the preserving solution, despite every precaution. Neutral formalin in some weak concentration, which has been experimentally determined for the species in question, is usually the best fixative.

Beroe ovata and young specimens of *Cestus* may be killed in chrom-osmic mixture, in which they should remain from fifteen to sixty minutes, according to size, and should then be transferred gradually to 70 per cent alcohol. While *Beroe ovata* is hardening in the alcohol, insert a short glass tube of the proper size into its gastric cavity to keep it distended in natural shape. Fix the tube so that it acts like a float to keep the animal suspended in the liquid. This operation must be effected with great care to avoid injuring the longitudinal series of vibratile plates. After one or two days, when the animal is in the 70 per cent alcohol, the tube may be removed and the hardened animal will retain its form.

Beroe forskalii, to be preserved in a state of expansion, must be immersed in the copper sulphate and sublimate mixture, and as soon as dead must be placed in the chrom-osmic mixture to harden for an hour. Since this species is naturally flat, it is not necessary to introduce a tube into it.

PLATYHELMINTHES

The Flatworms

The Collection of Flatworms

THE biological collector's chief interest in flatworms will probably lie largely in the securing of the parasitic species, flukes and tapeworms, which abound in all three of our great animal habitats, namely, terrestrial, fresh water and marine. There are, however, certain of the free-living flatworms that are of great importance in biological courses and of these, the species of planaria probably head the list.

TURBELLARIA

Planaria may be collected in small numbers by picking or washing them from the under side of leaves, stones, water-soaked boards, etc. Great numbers of *Planaria dorotocephala* Woodworth, and possibly other species as well, may be secured by placing small pieces of fresh meat (beef or liver) in water known to be inhabited by the planaria. The animals will gather upon the meat in numbers and may then be lifted from the water with the meat and washed off in a pan. A good method of collecting *Planaria maculata* Leidy is to bring into the laboratory from the pond or lagoon where the animals live, a large amount of vegetation (*Elodea, Myriophyllum, Potomogeton*) placing it in excess in an aquarium half full of water. As the water stagnates, the planaria which have been living on the vegetation will crawl onto the sides of the aquarium, forming a line just beneath the surface of the water. From here they may be scraped away in numbers. Related to the free-living planaria are the *Limulus* planaria, of the family *Bdellouridae.* This family contains three species, all parasitic or semiparasitic on this xiphosuran as it occurs along the east and south coasts of the United States. The *Limulus* planarian is usually secured by picking it from the gill plates of infected Limulus.

TREMATODA

Parasitic *Trematoda* must be collected from their specific host animals. *Fasciola hepatica* is more often found in the livers of sheep than in other domestic animals. It is endemic in southern Texas, where a considerable proportion of the sheep are infected, and may be secured from slaughter houses in that region.

Sporocysts, rediae and cercariae of flukes may be collected in the later summer from pond snails. The genus *Lymnaea* is most commonly infected, although *Planorbis* and *Physa* are by no means immune. In a single pool along the margin of Wolf Lake, in the Chicago region, the author has for years collected snails which showed an infection as high as 25%. To collect these stages from snails, the shells should be crushed and the bodies dropped into normal salt solution. The orange yellow to pure white sporocysts and rediae will be seen as they fall from the host tissue. Many cercariae will soon break from the rediae and, with a lens, may be seen in vigorous motion on the bottom of the dish. Fixation of this material is best performed with corrosive-acetic, followed by washing and upgrading to 70% alcohol.

Pneumoneces, of which there is a number of species, occurs in the lungs of frogs. The ordinary leopard frog seems to be most abundantly parasitized, some lots of frogs showing almost 100% infection. Other lots will be found in which the ratio of infection is very low, so that a considerable element of chance is involved in the collection of these worms. The cloacal contents of infected frogs is often very rich in fluke eggs and makes excellent class material for the study of these eggs. *Pneumoneces* is best fixed by flattening under a cover glass and immersing in Gilson's fluid.

The flukes of importance in human disease, such as *Clonorchis* and *Schistosoma* are completely unavailable to ordinary collectors. They must be secured from specialists who are working on these organisms.

CESTODA

Several species of adult tapeworms are available to the collector at practically all seasons of the year. Examinations of the intestinal contents of dogs and cats will show a goodly percentage of infections and, at intervals, individual animals will be found with the small intestines packed and even bulging with tapeworm parasites. The intestine of a highly parasitized dog may contain two or more species of tapeworms as well as the dog roundworms, which latter seem to be present in practically all dogs of the Chicago region. The tapeworms most likely to be found in dogs are *Dipylidium caninum* Linnaeus and *Taenia serrata* Goeze while *T. serrata* and *T. crassicolis* Rudolphi are common in cats. *Moniezia* Blanchard, the sheep tapeworm, can be secured at slaughter houses where sheep are being killed. Collections in late summer and fall are likely to yield the best results, although some worms are to be found throughout the year. The parasites in the case of all of the large tapeworms can be washed out of the lumen of the gut by placing one severed end of the intestine as a hose over a water spigot, or the intestine can be slit longitudinally and the worms washed or picked out with forceps. The worms should be separated out and killed as individuals before they are placed in the preservative, for otherwise as they die they will wind themselves into inextricable bundles of segments.

To obtain the smaller tapeworms, according to Megitt, the following procedure is recommended: Open the intestine in lengths of approximately 4 inches, one portion at a time, cut off the part opened and shake vigorously in a flat dish (a convenient size is 10 x 6 x 2.5 inches) filled with tepid water of approximately 40° C.; if the intestinal contents are not fresh and consequently contain much mucus, a slightly higher temperature should be employed. Salt solution or cold water should never be used. Worms which have floated away from the intestine may easily be seen in the water, often moving vigorously. On no account should they be separated by force as rupture of the strobilus with consequent loss of the scolex usually results. After washing in this manner, the intestine with the attached worms should be removed to another similar dish and examined with a powerful lens, preferably a binocular, the scolices and smaller worms being dissected out with needles. This process should be repeated with each portion of the intestine and, with care, will ensure the collection of 90 per cent of the cestodes. The writer has found that scraping the intestine with the back of a scalpel into a glass jar with subsequent washing, is both tedious and ineffective, entailing much additional work and usually causing the fragmentation of each strobilus; the scolices in particular are often detached and, in the case of the smaller forms overlooked. Examination under a magnification of 50, drop by drop, of the residue obtained after washing, has never increased the number collected to any appreciable extent. By the first process at least five times as many may be obtained in a shorter time and a more perfect condition. Unless very accurate cytological work be intended it is always best for the worms to be left in the water for a short time to allow of the relaxation of the musculature.

NEMERTEA

Cerebratulus is best known of our nemertean worms, the early developmental stages being of importance as typical for certain of the lower invertebrates. *C. lacteus* Leidy is said to be common near the low water mark from Maine to Florida, breeding from March to July. Collectors also report finding *Cerebratulus* in considerable numbers in the mud flats near Nahant, Massachusetts. The younger specimens are extremely tender and cannot be safely handled while the older worms are much tougher and will stand considerable pulling.

The Preservation of Flatworms

The flatworms are, for the most part, tough and strong enough to withstand comparatively rough handling.

TURBELLARIA

When these worms are not quite thoroughly extended in a little water they may be killed with boiling saturated sublimate and at once poured into a larger receptacle containing fresh water, where liquid and animals are allowed to cool. From this mixture transfer to fresh water, and after a few minutes to alcohol. For certain polyclads it is necessary that the sublimate be warmed a little again, otherwise the bodies break up.

Planaria may be allowed to become partially extended on a glass plate and then killed with Gilson's fluid or with hot corrosive sublimate. The animals flatten out almost perfectly by this method. Liver flukes may be killed with formalin after being flattened between glass plates without any preliminary anesthetization. All specimens killed between glass surfaces should be removed as soon as hardened as they will stick to the glass if left indefinitely.

CESTODA

Tapeworms may be killed by laying the live worms in extended condition on blotting paper saturated with Bouin's solution or formalin. The specimens if to be used for laboratory purposes can be removed for permanent preservation to formalin or alcohol after about thirty minutes hardening. Another method of handling the larger tapeworms is to pick up each worm by the larger end, i.e. the end away from the scolex, and allow it to hang down until its own weight causes it to stretch, when it should be dipped quickly several times into a jar of the fixing fluid, e.g. cold saturated sublimate. Smaller species may be drawn across the edge of the vessel after each dipping into the killing fluid to aid in the stretching and minute forms should be pipetted directly into the fixative. For tapeworms all fixatives with the exception of alcohol should be used cold. Zenker's fluid is considered by many to be the best fixer, but this fluid should not be allowed to act for more than 24 hours and subsequent prolonged washing is necessary. Bouin's fluid requires even more washing, but the material need not be removed so promptly, a factor which is important where laboratory facilities are limited, as in the field.

NEMERTEA

Great difficulty will be encountered in dealing with the nemertean worms, because before they are completely extended they may contract again, twisting the body badly and breaking to pieces. The nemerteans may be narcotized successfully, however, in a solution of chloral hydrate in sea water (0.1 of 1 per cent strength), where they should be allowed to remain from six to twelve hours and then hardened in alcohol in a long zinc box with wax in the bottom. When these animals have been narcotized for not too long a time in the chloral hydrate they will fully regain vitality and power of movement after a little if placed again in sea water. Another method which may be used in the expansion of nemerteans is to immerse them for 24 hours or more in a weak alcohol solution and, after they are completely anesthetized, to lay them out and fix them with formol-acetic alcohol.

By these methods good preparations can be made of *Cerebratulus* and other species in a state of perfect extension and with the proboscis protruded. The more resistant forms (*Amphiporus*), after they have been narcotized in a 0.1 of 1 per cent solution, may well be placed for some hours in a 0.2 of 1 per cent solution before they are killed. The larval form of *Cerebratulus* (the pilidium) is killed either with the copper sulphate and sublimate mixture or with saturated sublimate alone.

NEMATHELMINTHES

Roundworms

LIKE the flatworms, roundworms are to be found both free-living and as parasites upon other animals, while a few roundworms live in the tissues of plants. Free-living roundworms are usually small, but in number of individuals and variety of habitat they rival any of our most widely distributed animal groups, for example the Arthropoda, for roundworms are abundant in soil, fresh water and the sea.

The roundworms most widely used in the biological laboratory include a few species only. Most of these are parasites in warm blooded animals.

The Collection of Roundworms

Ascaris lumbricoides Linnaeus, may be secured in large numbers at any killing yard where pigs are being slaughtered. The live worms can be removed to the laboratory in a bucket where they should be preserved promptly. *A. megalocephala* Cloquet is to be secured from the horse intestine. Fixation and preparation may be the same as for *A. lumbricoides*, but in case the material is wanted for cytological work special methods must be used. *Echinorhynchus* Muller, the acanthocephalan worm from the pig, may also be preserved successfully according to the ascaris method.

Trichina—In certain localities wild rats show a high (90% to 100%) rate of trichina infection and laboratory material may be secured by trapping them. Rabbits, cats, dogs or rats may be infected artificially from rat material and the adult worms secured by killing the experimental animal at the proper time. Small amounts of trichina infected muscle can sometimes be secured from local slaughter houses providing the proper local and governmental permits have been secured. Muscle containing the encysted larvae should be dropped into 50% glycerine. Change the glycerine two to three times and store in the same. The glycerine treatment macerates the muscle fibers and keeps them soft and pliable.

According to Christenson, intestinal trichinae may be recovered by the following method. A rat is starved for two days and is then fed trichinized tissue. Before the animal is killed it is kept unfed for a day to eliminate the vegetable debris. It is then killed with ether and the intestine removed to a normal saline solution. The intestine is then taken, a short section at a time, and its contents stripped into a vial containing normal saline. This can easily be done by holding one end of the intestine above the vial with one pair of forceps and stripping towards the free end with another pair. The intestinal contents issue from the lumen of the free end and are thus concentrated in the bottom of the vial. The intestine is not slit lengthwise as has been done in the other methods. After the entire intestine has been stripped in the manner detailed above, the extraneous materials can, in a large part, be removed by screening the worms through a 40-mesh screen. To insure complete success of the method the screenings should be examined microscopically, especially if there is any great amount of debris left. By having the water level in the container above the lowest point of the concavity of the screen the debris is not allowed to settle in a compact mass from which the worms cannot crawl.

If larval trichinae are desired they may be recovered by taking some of the tissue of a rat infected for 16 to 20 days and tearing it apart in normal saline. The diaphragm is especially suited for this, although the tongue, masticatory, and neck muscles do very well. Well formed cysts are found after the infection has run from 30 days. Encystment begins at about 20 days after infection, but the cysts are not well formed for a short time later. Calcification of the cysts results if the infection is allowed to run for eleven or twelve months.

The *Gordiidae* are the hairworms (hair-snakes) to be found in sluggish streams, ponds, drinking troughs, open cisterns, etc. in late summer. The body (3-5 inches long) resembles in appearance a bit of fine wire or a coarse black or brown hair from the mane or tail of a horse. The anterior end is usually rounded. The posterior end of the male is often modified into a swollen lobe or may be curled in a loose spiral. *Gordius* Linnaeus is one of the most common of the hairworms; the sexes in this form are distinctly unlike and the sexes of the adults may usually be distinguished by their color, the males being blackish brown and the females much lighter. The males are about a third smaller than the females. During the winter months the hairworms are inhabitants of the soil and may be secured by digging among the grass roots along the banks of small streams.

The Preservation of Roundworms

Because of the tough cuticle in which all roundworms are enclosed, killing fluids do not penetrate readily and therefore it is best to do the killing with hot solutions so that the worms may be rapidly killed by the heat if not by the solution. The horse or pig ascaris for example, will itself die fairly quick in a 10 per cent solution of formalin at room temperature, but the eggs inside will continue to develop until complete worms are formed inside the shell. These tiny worms may remain active for weeks although immersed all the while in the formalin solution. Also, the larvae of certain species of *Mermis* Dujardin will live and remain active in cold 10 per cent formalin for days or even weeks. Because of this high impenetrability of the roundworm cuticle, it is customary to kill the worms in hot formalin, for roundworms, as do most of the cold blooded animals, show little resistance to even moderately high temperatures, often dying quickly in solutions whose temperature is no more than slightly uncomfortable to the hands.

Ascaris lumbricoides Linnaeus is best killed by the hot-water method. Heat water until it is just below the boiling point, lay out several dozen worms in a piece of cheesecloth, grasp the bundle at each end and immerse the extended worms in the hot water for just a moment. Then remove them and lay them out in a pan of five per cent formalin solution.

In general, most of the smaller nematodes, free and parasitic, may be killed with saturated sublimate or with Kleinenberg's liquid and stored in 70 per cent alcohol.

TROCHELMINTHES

Rotifera or Wheel Animalcules

The Collection of Rotifers

If plant material from a lagoon or pond be crowded in a jar or pan with very little water, the rotifers will come to the surface film in numbers. Rotifiers are also commonly found in hay infusions and other mixed cultures, while they may themselves be grown in pure cultures if this is desired.

The Preservation of Rotifers

An excellent method for the preserving of rotifers is as follows: Make up a stock solution of cocaine hydrochlorate, 1 gram, water 50 c.c., and alcohol 12 c.c. This solution will keep indefinitely. To use, dilute the stock solution in the proportion of two volumes of stock solution to three of water. If this dilute solution be added slowly to the dish in which the rotifers are contained they will be gradually anesthetized. When the cilia have ceased to move the animals can be killed in extended condition by adding to the solution a drop to a few drops of one per cent fresh osmic acid solution. After washing in water, permanent preservation is best made in ten per cent formalin solution.

CHAETOGNATHA

Sagitta may very well be treated in the mixture of copper sulphate and sublimate or in the chrom-osmic mixture after which they are stored in 70 per cent alcohol.

SIPUNCULOIDEA

The Preservation of Sipunculoidea

Sipunculus may be killed with chromic acid, one-half of 1 per cent or even weaker, in which the tentacles usually, but not always, expand before death. The animal after being narcotized with chloral hydrate of 0.1 of 1 per cent in sea water, dies with tentacles extended. Both methods are good, but sometimes a portion of the animal remains contracted, and sometimes during the process the skin in front breaks and allows all the perivisceral liquid to escape, resulting in distortion of the body. *Phascolosoma* may be placed in alcoholized sea water and allowed to remain there until dead (three to six hours).

With the large specimens of *Bonellia* it is best to wait until the proboscis has become well extended and then seize the body of the animal with one hand and the extremity of the proboscis with a pair of forceps so that it can be kept extended. Then quickly immerse the whole in Kleinenberg's liquid in a wax-bottomed tray, keep the animal stretched out to prevent contraction and wait until it dies. After an hour in this solution the transfer to alcohol may begin. Small bonellias are narcotized in alcoholized sea water and fixed in weak alcohol.

PHORONIDEA

**Phoronis* is allowed to remain half an hour in alcoholized sea water and then is killed with boiling saturated sublimate.

---

*Classified by some authorities with Lower Chordates.

MOLLUSCOIDEA

THE *Bryozoa (Polyzoa)* are mostly of minute size and usually colonial in habit, growing attached to rocks, plants and other objects. The larger number live in sea water, but they are not uncommon in fresh water. The *Brachiopoda* have a body protected by a bivalve shell and are of fairly large size. With the exception of the burrowing *Lingula*, the brachiopods live attached to rocks and other solid objects.

The Collection of Molluscoidea

Bugula is to be found growing attached to sea weeds in tide pools which are drained dry by only the lowest tides if at all. Upon first sight, the reddish brown growth may be mistaken for a hydroid since the growth habits of *Bugula* and certain of the hydroids are similar. Examination will remove any doubt, however, for the characteristic growth form of the colonies and the movements of the individual animals as seen under a lens will identify them beyond a doubt.

Bugula may be collected in quantity at low tide and if kept in fresh sea water will live for some hours even though the water is not changed. Death is indicated by discoloration of the water by a bright purple pigment dissolving into the water very soon after the animals die.

The *fresh-water Bryozoa* can be secured during the summer months from ponds and streams where, as in the case of *Pectinatella*, large spherical jelly-like masses grow loosely attached to plant stems or other objects in the water. *Plumatella* Lamarck forms plant-like growths on the rocks or may attach itself to the vegetation, being easily collected in the latter position. It is often common on the undersides of flat stones in small swift streams, where it may be found until late fall.

The *marine Bryozoa* of shallow water may be collected with ease on almost any coast. The fronds of the sea-mat, *Flustra foliacea* Linnaeus are thrown up by the waves in thousands along coasts where the water is shallow and the bottom sandy. The leaves of the kelp, *Laminaria*, are frequently more or less covered with delicate lace-like encrustations which upon investigation prove to be *Bryozoa*, and a further type builds up a beautiful, branching, calcareous, coral-like skeleton upon a rock or sunken timber.

The Preservation of Molluscoidea

The *Bryozoa*, or moss-animals, are exceedingly difficult to preserve in extended condition as they do not react well to anesthetics. The author has had very good success with *Bugula* by placing clusters of the animal in finger bowls in the smallest amount of sea water that will cover the colonies. If the bowls are now set in the bright sunlight the animals soon expand and can be killed in nicely expanded shape by suddenly flooding them with boiling hot Bouin's fluid. *Pectinatella* and *Plumatella*, the fresh-water bryozoa, can be killed extended in this same way although the growth habit of these two types may offer technical difficulties that must be overcome. With *Pectinatella*, small portions of the colony must be detached and treated as above.

The Biological Station at Naples, Italy, reports the following methods for the *Bryozoa:*

Pedicellina and *Loxosoma* should be left for an hour in chloral hydrate 0.1 of one per cent and then killed with saturated sublimate, cold or hot. Wash immediately afterwards and place in alcohol. Certain species of *Bugula (purpurotinota, turbinata)* after the animals have become well extended in a little sea water, are killed quickly with hot sublimate. By pouring some 70 per cent alcohol slowly over the surface of the water in which they are, *Flustra, Cellepora, Crisia, Bugula* and *Zoobotrum* have been prepared in a state of complete extension. The other species can be killed with the animals more or less out of their cells by using a weak solution of chloral hydrate or alcoholized sea water, but generally good results depend upon the skill of the preparateur.

The *Brachiopoda* are not abundant along our coasts and therefore we know little of the details of their preservation. We have preserved *Lingula* and *Terebratulina* D'Orbigny by dropping them directly into 10 per cent formalin. Some technicians report that brachiopods may be narcotized by letting them lie in alcoholized sea water for some hours before they are put into alcohol. With the larger species a bit of cork may be placed between the valves to keep them open. Small forms may be put at once into 70 per cent alcohol.

ANNELIDA

Annelid Worms

THE annelid worms are well represented by marine, fresh-water and terrestrial forms. The most common of these, from the laboratory standpoint, are *Nereis* (marine), *Lumbricus* (terrestrial) and *Hirudo* (fresh-water).

The Collection of Annelid Worms

The majority of the annelid worms known to laboratory workers are marine forms which spend all or part of their existence in the mud of the bottom. The earthworms, however, are largely terrestrial, while many small annelid species inhabit the mud and humus of the bottom of fresh-water streams and ponds.

From the burrowing habit of the annelid worms it will be seen that their collection is likely to resolve itself into a matter of digging up the soil and separating the animals from it and frequently this is the method to which the collector must resort. However, in the case of certain of the species most sought after, some quirk in the behavior of the animals brings them temporarily within the power of the collector, who if he luckily be present at that time, may reap a rich harvest with comparatively little labor.

CHAETOPODA

Nereis virens may be secured in quantity by netting them at the time of the mating swarm. They may also be collected by hand digging on the sandy ocean shores at very low tides. The worms as they are uncovered must be captured quickly or they will disappear again into the sand. Dredging operations will at times bring up large numbers of Nereis.

Lepidonotus (*Polynoe*)—Along the water line at low tide one may secure the scale worm in quantity by turning over the loose rocks. The number of species is considerable and their coloring varied. Close observation is essential upon the part of the collector, as the worms have a habit of remaining quiescent on the surface of the uplifted stone and may easily be overlooked.

Lumbricus—In the Chicago region, Earthworms (*Lumbricus terrestris* Linnaeus) may be collected in quantity on rainy nights during the spring and fall. In summer the rains are likely to be short and hard followed by quick drying, giving the worms little opportunity to emerge. The best collecting season is in the spring. During the winter the worms are confined below the frozen earth, but with the spring rains and the thawing of this frozen crust they swarm to the surface. In the Chicago region this swarming usually occurs during the month of April at which time spells of rainy weather of two to three or more days duration are common, with the precipitation heaviest during the night. It is during the time of these cold spring rains that the earthworm collector equipped with rubber boots, raincoat, bucket and flashlight repairs to the shrubbery in the park at dark. On hands and knees he moves cautiously through the undergrowth and with straining eyes endeavors to distinguish the worms from the twigs and leaves with which the ground is covered. As visual adjustment comes, the worms may be seen lying with the anterior half of the body out of the hole. By a quick dart of the hand the worm is seized just where its body emerges from the hole and with a steady pull the posterior end is drawn out and the worm dropped into the bucket. Should the pull be too vigorous the collector feels a sudden giving way and finds himself holding a half worm, the other half being securely anchored in the hole. It is the collector's hope that the rain will continue regardless of its chilling effect and despite the fact that the ground over which he must crawl is becoming muddier and muddier, for should the rain cease, the worms soon retreat into their holes. Earthworms must be stalked with some care, for they are sensitive to the jar of the earth caused by the approaching collector and will jerk back into their holes like a flash at the faintest vibration of broken stick or incautiously placed hand, knee or pail. Should the rain pour down in a steady, chilling sheet, the collector picks worms with both hands and, with continuous rain, many of the worms leave their holes entirely. Copulating pairs are now numerous and two worms can be scooped up with a single motion. Should the rain continue all or most of the night and into the next morning, the streets and roads may become covered with worms that have crawled or been washed onto the hard surface and are unable to escape by burrowing. With the sunrise these worms will disappear, however, for with a few minutes of sunshine the epidermis of the worms that have not been able to burrow becomes so sticky that they soon become covered with all manner of dust and gravel particles and are scarcely distinguishable from the soil.

Various chemicals have been used to drive earthworms from their holes in soil where they are not desired. A solution of mercuric chloride is recommended by some of the seed houses, it having been found that spraying such a solution on golf greens will cause the worms to crawl to the surface and die. The use of such solutions in collecting may offer possibilities.

HIRUDINEA

Leeches may be secured in spring, summer and fall by use of the dip net. Vigorous swishing about in and through the vegetation of ponds will secure a number of species. They may also be secured by hand, picking them from their aquatic hosts. The animals will be found abundantly in sluggish to stagnant pools, rivers or lakes, where plenty of aquatic vegetation is present. Such forms as *Clepsine* may be found on the under side of floating boards or logs or under submerged stones; *Glossiphonia* (*Clepsine*) is also a common inhabitant of the outer shell of snapping-turtles where it seems to live without much regard to the turtle as a food source. Leeches of the *Placobdella* type are common in frog pens in the spring months, while a number of species of leeches may be secured from hibernating turtles throughout the winter season. Considerable business is done in the sale of leeches for pseudomedicinal purposes, many thousands of large specimens being imported into this country from France and Italy each year.

The Preservation of Annelid Worms

A group so varied in structure and make-up as the annelid worms is bound to require variation in treatment by the preparateur. Many of the marine annelids can be preserved directly in ten per cent formalin when extended proboscides and appendages are not required. When fully expanded specimens are needed, anesthetization must be resorted to.

CHAETOPODA

Many of these worms, if placed in a fixing fluid which is too energetic in action, contract greatly and twist out of shape and many of them break to pieces, so that an idea of the natural form is lost. This trouble may be obviated by very gradually pouring over the surface of the sea water in the crystallizing dish a stratum of a mixture of glycerin, one part, 70 per cent alcohol, two parts, and sea water, two parts. This stratum will slowly diffuse throughout the water and after some hours the animals will be narcotized and will remain fully extended, if transferred to alcohol. Experience has shown that alcohol alone may usually suffice for the treatment of these worms for instead of the mixture just described, one may add to the sea water five per cent absolute alcohol and let the animals remain therein until they have lost motion, an operation which varies from two to twelve hours for the different species. It is a good plan not to allow these worms to become entirely dead in the sea water. The hardening should be done in long wax-bottomed trays with 70 per cent alcohol, straightening out the animals and holding them in place when necessary by means of wooden pins. After a few hours in the tray, they should be put into tubes and allowed to rest in a horizontal position for a day or so. Since 70 per cent alcohol does not penetrate the tissues of these animals well enough to prevent maceration, 90 per cent alcohol must be used for permanent preservation. Large specimens should be suspended in the tube by means of a thread attached to a float.

The pelagic larvae of *Echiurus* are well fixed by allowing them to lie for some minutes in the mixture of sulphate of copper and sublimate.

Lumbricus—Once the earthworms are collected, they may be prepared for class dissection by placing them in pans of water and with a dropping arrangement allow ethyl alcohol to drop into the water. They can be anesthetized in about an hour by adding sufficient alcohol to the water to make a ten per cent solution. Properly anesthetized the worms may be laid out in a long pan and covered with a solution of five per cent formalin, which solution will both kill and harden the specimens, which may then be preserved in a fresh solution (about five per cent). To prepare worms for histological work collect sizes and ages desired, feed them for a few days on lettuce, agar-agar or fresh meat and, after anesthetizing, kill in Gilson's or Bouin's fluid, cutting the worms into short pieces (one-half inch to one-inch) before dropping into the killing fluid. See a text on microscopic technique for further procedure.

HIRUDINEA

Leeches can be anesthetized in warm water in chloretone or in magnesium sulphate solution, or they may be asphyxiated in a tightly closed jar of water and then injected with ten per cent formalin to extend them. Properly preserved leeches appear quite lifelike.

Leeches may also be killed in chromic acid of one-half of one per cent or hot saturated corrosive sublimate solution. In any case it is well to straighten and harden in a wax-bottomed tray.

ARTHROPODA

CRUSTACEA

THE *Arthropoda* of this class are represented in all three of the great earth habitats, although the terrestrial crustacea are by no means so important in numbers or size as are those found in the water. The various species of crayfish make up the bulk of the fresh-water crustacea, although certain of the smaller forms exceed them in numbers. The real home of the crustacea, however, is the sea, for here their abundance almost equals that of the insects on land.

The Collection of Crustaceans

In general, *Crustacea* are taken either by hand or in traps although the smaller forms may be procurable by netting only. Practically all plankton towings in either fresh or salt water will contain large numbers of *Entomostraca*, while a certain number of crabs, crayfish, etc. are taken by fishermen in hauling their seines. The crayfish and lobster trap is a loosely built square or oblong crate with an opening on one or two sides where the animals may crawl in to the food (bait) which is located within. Once in, the trapped animals seem unable to find the entrance hole and so remain in the trap until removed by the owner who hauls the trap to the surface at intervals of a few hours to a day or more.

Branchipus Schaeffer may be collected in quantity by straining the water of pools in which they occur. On sunny days the semi-transparent animals are readily distinguishable in the shallow water and a dip net will soon confirm the observation. Since the organisms are exceedingly fragile, one must proceed cautiously to secure perfect specimens. By dipping them carefully from the water and by using great care in transferring them without handling to the preserving jar, perfect specimens free from all debris may be secured. Living specimens brought to the laboratory in a Mason jar full of the pond water will furnish an interesting addition to the balanced aquarium. Undisturbed in such an aquarium they will live for days or perhaps weeks, if collected during the first of the spring days. It should be remembered, however, that such specimens can be taken for a brief period of time only in the spring season. Preserve in 70 per cent to 80 per cent pure grain alcohol.

Daphnia—The bottom of a dried-up pond may be richly seeded with ephippia containing the winter eggs and if a handful of such soil be placed in water, an abundant crop of Daphnia will likely result.

The ordinary means of collecting other forms of small water animals and plants will serve for *Copepoda*. A tow net, or a fine-meshed dip net will suffice. Care exercised to secure the animals as free from debris as possible will save much time in the end. Seventy-five per cent alcohol is the best preservative, although 5 per cent formalin will do well enough for most purposes.

Barnacles can be pried from their attachments and preserved in 10 per cent formalin. To preserve with appendages extended they must first be anesthetized with magnesium sulphate or other anesthetic.

Lobsters are captured in traps (lobster pots) made of slats and baited with fish heads or other animal refuse. By law the taking of small lobsters of laboratory size is prohibited except by special government permit. The animals are killed by immersion in fresh water and are preserved by injecting 5 per cent formalin into the stomach and body cavity.

Crayfish are collected in traps and nets for the most part. The burrowing species may at times be caught out of their holes, especially during the rainy season; otherwise they must be dug out. By digging deep enough (one to three feet) the pocket in which the crayfish lives will eventually be reached.

Preservation is best accomplished in pure grain alcohol. Good results are attained by first killing the specimen in a rather weak solution (50%) and then transferring through two or three changes to 75 to 80%. When so treated, the appendages should remain soft and flexible as in life. When alcohol is not available preserve in 8 per cent formalin. Inject some of the preservative into the body cavity.

Hermit Crabs occupy empty snail shells and a rapidly moving snail shell is a good indication of the presence of a hermit crab. It is usually necessary to break the shell if one wishes to remove a hermit crab in haste, for so firmly are they anchored that the abdomen will likely be left in the shell if force is applied. Dropping the inhabited shells into warm water will cause some individuals to desert their shells and in the case of smaller species, at least, crowding in too little water will result in a large proportion of them leaving their shells within a short time.

Fiddler Crabs may be dug up, captured on the beach, or driven in swarms into pitfalls. Since their only economic importance is to provide fish bait they are not molested to any considerable extent.

The Preservation of Crustaceans

In addition to the methods outlined above, the following special notes may prove helpful to the collector.

The marine *Cladocera (Podon, Evadne)* are killed with saturated sublimate, or with a few drops of osmic acid of 1 per cent in the sea water containing them, removing them when they begin to turn brown. Wash and put into 70 per cent alcohol.

Ostracoda are put at once into 70 per cent alcohol.

Copepoda—The free forms are killed in a saturated solution of sublimate in sea water, where they are allowed to stay from five to ten minutes. The parasitic forms may be killed in the same way or be put at once into weak alcohol.

Cirripedia—To prepare *Lepas, Conchoderma,* etc., with appendages extended, let them die in alcohol of 35 per cent. If the appendages of certain species contract they can easily be drawn out again by means of forceps.

Balanus and similar forms are immersed directly into alcohol of 70 per cent, taking care to change the liquid soon.

Rhizocephala (Sacculina, Peltogaster, etc.) are placed for fifteen minutes in a mixture of 90 per cent alcohol and sublimate in equal parts, and then transferred to 70 per cent alcohol.

Amphipoda were formerly prepared by putting them at once into alcohol of 70 per cent, but now they are put into Pereny's solution for fifteen minutes before they are immersed in the alcohol. The transparent forms of the last division (*Phronima,* etc.) are killed in sublimate.

Isopoda—These are put at once into 70 per cent alcohol, with the exception of the *Bopyrides* and *Entoniscides,* which are first placed in a mixture of 90 per cent alcohol and saturated sublimate in equal parts (like the *Rhizocephala*) or in saturated sublimate alone.

Cumacea and *Stomatopoda* go directly into alcohol, though the transparent larval forms of the latter are first put into saturated sublimate for a few minutes.

Schizopoda go at once into alcohol or they may be treated first with saturated sublimate.

To avoid the breaking of the appendages of the *Decapoda,* these forms are allowed to die in fresh water before they are put into alcohol. They remain in fresh water only as long as is necessary, otherwise the membranous appendages are injured.

With the *Paguridae* the alcohol must be changed often, and they are to be preserved permanently in 90 per cent alcohol, because the shell is permeable to only a small degree.

The larvae of the *Decapoda* (*Zoea, Phyllosoma,* etc.) are killed in sublimate or with a few drops of osmic acid of 1 per cent.

ARACHNOIDEA

The arachnids are, with a few exceptions, terrestrial forms, there being but a few mites and spiders in fresh water while the only representatives of this class to be found in the sea are certain insignificant, inconspicuous species of the group *Pycnogonida,* that occur among the vegetation along the ocean shore and *Limulus,* the horseshoe crab.

The Collection and Preservation of Arachnids

The collecting of the land arachnids is almost entirely a matter of securing them one at a time by hand, for their solitary habit and rather fragile bodies render any other method impractical.

Collecting the large female garden spiders is easily accomplished by seizing them in the fingers and dropping them into a jar of alcohol, or for those who prefer not to touch the animals, capture may be accomplished by holding the jar beneath the spider and raising it till the edge of the jar opening strikes the web. Usually the spider will at once drop straight down into the jar, their only method of escape seeming to be to drop suddenly to the ground beneath the web, there to lie inert or perhaps to crawl away into some crevice.

Most spiders may be preserved satisfactorily in 70 per cent ethyl alcohol, although the fleshier forms intended for internal dissection will be most satisfactory if preserved in Kahle's solution and then transferred to 70 per cent alcohol.

Limulus is preserved by being dropped into 8 per cent formalin.

Acarina

With few exceptions mites and ticks are parasitic, chiefly on animals, although a few species of mites (of the family Eriophyidae) produce galls on plants. Their collection, therefore, is largely a matter of finding animals infected with the particular species wanted. *Trombicula irritans*, the common North American chigger is widely distributed throughout the southern states. Larvae may often be collected in large numbers by allowing a dog to walk through moist vegetation and then brushing the mites off onto white paper. They should be preserved directly in alcohol of about 70 per cent. *Tyroglyphidae* feed on decaying organic matter. The most common is the cheese mite which is often to be found in very large numbers on old cheese. *Sarcoptidae* include some of the most troublesome parasites of man and domestic animals. *Chorioptes equi*, the foot mange mite of horses; *Notoedres cati*, the head mange mite of cats; *Psoroptes ovis*, the scab mite of sheep; *Sarcoptes equi*, the scab mite of horses and *Sarcoptes scabiei*, the human itch mite, are all important economic forms whose collection is self explanatory with the naming of the host. For laboratory material the most easily obtained sarcoptid mite is *Notoedres cati* or *Psoroptes cuniculi*, the ear mange mite of rabbits. *Demodex canis*, the dog follicle mite, causes the very severe red mange of dogs. The mites may be secured in quantity by squeezing the hair follicles of infected dogs.

The most important ticks and possibly the most easily obtained are: *Argas persicus*, an important chicken pest; *Rhipicephalus sanguineus*, the common dog tick; *Margaropus annulatus*, the North American cattle tick, transmitter of Texas fever; *Dermacentor andersoni*, the transmitter of Rocky Mountain spotted fever.

All ticks and mites may be killed by dropping them directly into 70 per cent alcohol.

MYRIAPODA

The centipedes and millipedes are very widely distributed, but the number of species is not large. They are most often to be found under logs or stones. In warm climates and in the latter part of the summer they may be secured in large numbers with relatively little work. They should be preserved in Carl's solution. Due to the hard shell possessed by these animals, it is desirable to inject some of the fixative into the body cavity with a syringe.

INSECTA

Insects are extremely plentiful and the capture and preservation of at least a few of the common forms is a part of the field collector's summer work. Many forms are of great economic importance and about a dozen types are very generally used for study and dissection in beginning biology courses. No attempt can be made in this booklet to cover the entire field of insect collecting and the teacher interested in this is referred to the many excellent entomological texts available in any public library. The following suggestions are general and necessarily subject to modification to meet special collecting conditions.

Types of Collecting Nets

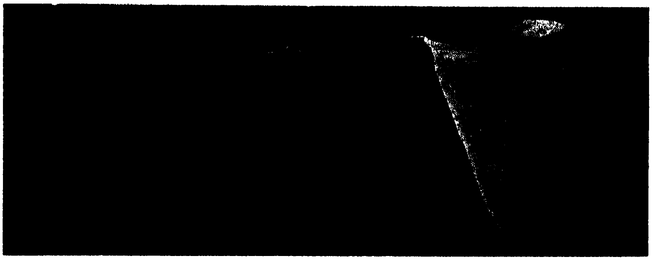


Fig. 1

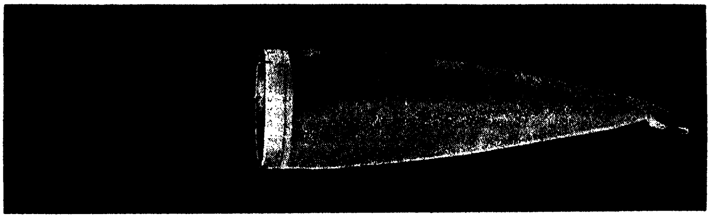


Fig. 2

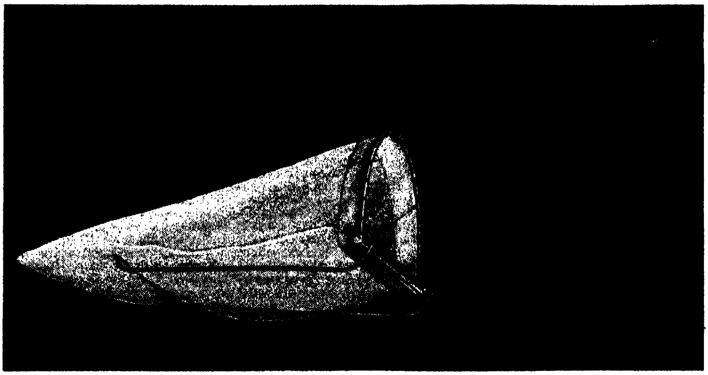


Fig. 3

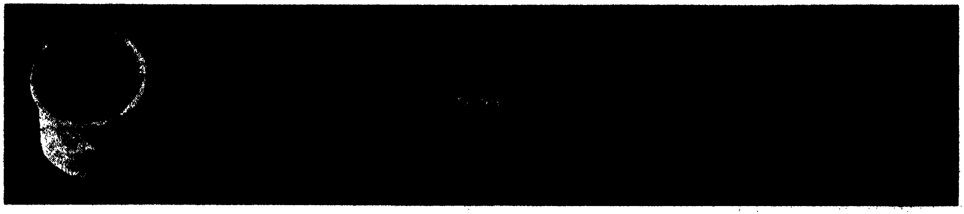


Fig. 4

Fig. 1. Air net for collecting flying insects.
Fig. 2. Towing net for taking plankton from ponds or lakes. May also be used with a handle for collecting from banks of streams.
Fig. 3. Dredge net for bottom collecting from fresh or salt water.
Fig. 4. Long handled dip net. Used in taking aquatic forms from ponds and streams.

All of the nets illustrated here are manufactured by The General Biological Supply House, Chicago, Ill.

The Collection of Insects

General collections of the more common local insects can be made by means of random collecting from time to time throughout the summer season. The collecting of large numbers of a given species and particularly the securing of complete life cycle sets of a certain insect depends, however, upon rather exact knowledge on the part of the collector. To do this work successfully the collector must be familiar with the life history, the feeding habits and the type of habitat frequented by the insect he is seeking. Much time can be saved by knowing in advance the season when certain insects occur most abundantly and the places where they may be expected in the greatest numbers. Catching monarch butterflies in a net, for instance, is a slow and tedious task, but the securing of these same insects when they swarm in preparation for migration is a simple matter.

Collecting Equipment for the taking of insects varies greatly and is dependent upon the type of collecting being done and the kinds of insects desired. We have found certain baited and illuminated traps effective for securing insects in quantity, but here again, we must refer the collector to those texts dealing exclusively with Entomology. For general collecting the most essential tools are (a) an aquatic net, (b) an air net and (c) one or more containers in which to place the captured specimens.

Aquatic collecting should not be done with an ordinary air insect net; for this purpose a dip net of sturdy construction should be used. The net may be worked from side to side in the water or swept over the water weeds. In swift-flowing streams the net may be held against the bottom of the stream while the stones just above the net are moved. Many forms will float with the current and will be caught in the net. Some aquatic insects may be picked from the undersides of stones and sticks.

Terrestrial collecting may similarly be divided into various types. Many insects, particularly beetles, can be captured by moving stones or sticks or by digging them out of rotten logs. Others can best be collected by "sweeping." This is a term applied to collecting insects by passing the net rapidly back and forth over the grass and weeds. If much of this type of collecting is to be done, at least one sturdy sweep net should be used. Nets of various approved types are illustrated on the opposite page.

After the insects have been captured they must be killed and transferred to containers such as small boxes, vials or jars. Cyanide jars are commonly used to kill insects. Such jars may be purchased or made up as follows: Place about one-fourth inch of potassium cyanide in the bottom of the jar and cover with an equal amount of fine sawdust. Pour plaster of Paris over this. Cut three pieces of blotting paper of the same diameter as that of the jar. Place the blotting paper on the plaster and press it down firmly. It is well to have the top piece slightly larger than the others in order to make a firm fit. The jars should be kept covered when not in use. A few grains of calcium oxalate added to the cyanide produces a more effective killer, but it has the disadvantage of deteriorating more rapidly.

Since the potassium cyanide and its fumes are *deadly poisonous*, great care should be exercised, labeling the jars "Poisonous Fumes." They should not be left where children or people unfamiliar with the nature of their contents may have access to them. Due to the extremely poisonous nature of the cyanide it is more advisable to buy jars already prepared than to make them.

A very satisfactory non-poisonous killing jar which uses carbon tetrachloride instead of cyanide is preferred by many collectors and is recommended for young students. Such a jar was developed in 1931 by the General Biological Supply House and has proved to be entirely successful.

As soon as they are dead the insects should be removed from the killing jars and transferred to small boxes, vials or other suitable containers. Insects intended for pinning should be taken to the laboratory "dry;" specimens to be preserved for dissection or for permanent display in liquid may be dropped directly into alcohol or some other preservative without first having been killed in the cyanide jar.

The Preservation of Insects

Insects are usually collected for one of two purposes. First, the specimens may be desired for laboratory study and dissection, in which case they are usually preserved in liquid. Second, specimens intended for display in the permanent insect collection are usually pinned out in the regular manner and are "preserved" only by drying.

Preserving Insects in Liquid

In general it may be said that all of the larger insects with heavy chitinous exoskeletons, which are to be preserved for dissection, must be injected with preservative. If this same type of insect is to be preserved for external study only it is sufficient to drop the living specimens into the preservative. Enough solution will be swallowed and taken in through the spiracles to give a fairly satisfactory preservation.

The following methods are those which we have found to be most satisfactory in insect preservation:

1. Preservation with Kahle's solution or with Carl's solution.
2. Preservation with chloral hydrate.
3. Preservation with 80 per cent ethyl alcohol.

Kahle's solution and Carl's solution (see appendix for formulae) are both very satisfactory insect preservatives, the latter being the most useful. It penetrates well and at the same time keeps the tissues in a flexible condition, so that the appendages do not break or drop off. Smaller insects, such as bees, wasps, flies, etc., can be dropped into the solution and kept there for an indefinite period without deterioration. The larger insects should be injected with the solution, preserved in it for a few days and then stored in 70 per cent alcohol.

Fleshy insect larvae such as the Sphinx larvae may best be preserved by injecting Carl's solution into the body cavity and storing them in the same fluid. Alcohol and glycerine mixture is also used with good results. The injection of the body cavity is important for giving the specimens a natural, plump appearance as well as for preserving the internal organs.

For some forms used in insect morphology courses the chloral hydrate method seems to be superior. This is particularly true of the Dobson-fly larva, *Corydalus cornutus*, which is very commonly used for dissection purposes. An aqueous solution of chloral hydrate, made up of one part of chloral hydrate and twenty parts of water, is used. Immerse the living insects in this solution for one day. Then make small dorsal longitudinal cuts and two small ventral slits, one anterior and one posterior, to allow the preservative to penetrate. Change the solution each day for three days and then store in fresh solution.

Eighty per cent ethyl alcohol may be used for all ordinary preservation of small insects. Do not use denatured alcohol, as the denaturing ingredients render the insects very brittle. Large insects intended for internal dissection should have the alcohol injected into the body cavity. At no time during the preservation process should the insects be packed tightly into a jar. Crowding will result in distorted, broken and poorly preserved specimens.

For occasional use when internal preservation is not important insects may be dropped into rubbing alcohol, or into a solution of denatured alcohol to which a small amount of glycerine has been added.

Mounting Insects for the Display Collection

This subject is discussed in most Entomology texts and we therefore offer only a few suggestions for the beginner in this booklet.

Mounting Equipment. Insects collected in the field should, if possible, be mounted the same day. If allowed to dry before being pinned and mounted it will be necessary to relax them in a moist box.

Pinning Boxes. Cigar boxes do well for the beginner. These may be prepared by securely fastening in the bottom of each a layer of soft balsa wood or pressed cork. Corrugated paper may also be used. Four large cigar boxes furnish ample space for starting the collection. Standard insect boxes and glass-topped display cases may be purchased for reasonable sums.

Pins. Special insect pins should always be used in mounting. Number three is the most satisfactory size.

Cardboard Points. These are needed for mounting insects which are too small for pinning. These can be made with a special punch or may be cut from rather heavy glazed paper.

White Shellac. This is used in gluing insects to the paper points.

Pinning Forceps are useful in pushing pins into the cork, but are not absolutely necessary for the small collection.

Insect Forceps. Insects should always be handled with forceps. Some prefer the fine-pointed curved forceps and some the broad-pointed.

Labels. Labels can be made from good quality paper. Order and family labels should be outlined with black, while genus and species labels should be plain. The black border may be made by means of a drawing pen and India ink. Order labels should be two and one-fourth inches long by one-half inch wide; family labels one and three-fourths by one-half inch. Genus and species labels should be one and one-half by one-half inch.

Directions for Mounting. The method of mounting differs in different orders of insects, according to type of characters used in classification.

Orthoptera (grasshoppers, et al.), *Neuroptera* (dobsonflies, et al.), *Ephemeroptera* (May-flies), *Odonata* (dragonflies and damselflies), *Homoptera* (cicadas), *Mecoptera* (scorpion flies), *Trichoptera* (caddice flies), *Lepidoptera* (moths and butterflies), and *Hymenoptera* (bees, ants, and wasps) should be pinned through the middle of the thorax. In some of these it is best to put the pin a little to the right of the center in order not to destroy any structure which may be in the median dorsal line.

Hemiptera (bugs) should be pinned through the thorax (scutellum). *Coleoptera* (beetles) should be pinned through the right wing, a little less than one-fourth of the length of the wing from its base.

Diptera (flies) should be pinned through the mesothorax a little to the right of the center.

Many small *Hemiptera, Homoptera, Coleoptera,* and *Hymenoptera* must be mounted on cardboard points. The points should be triangular in shape and about one-fourth inch long and one-eighth inch wide at the base. These are conveniently made with a punch or cut from rather heavy, glazed paper. The specimen is glued to the point with white shellac. It should be on the left side of the pin when the head of the insect is directed forward.

Small *Lepidoptera* are pinned on minute pins fastened to a small piece of balsa wood or cork. A large insect pin is then thrust through the balsa wood and the specimen adjusted to the proper height.

Small *Diptera* may be mounted like small *Lepidoptera* or they may be glued to the pin by their right side at the same height they would have been if the pin were thrust through the body. Diptera should never be mounted on paper points.

In small collections, the larger butterflies and moths are best pinned with the wings slightly spread. Without specialized training in Entomology it is impossible for the average collector to identify many butterflies further than the family, so spreading is not necessary.

In the case of large specimens it is well to place a drop of shellac where the pin enters the insect. This holds the insect firmly on the pin and prevents the danger of its swinging around and breaking adjacent specimens.

Locality Label. This should be as small as possible and should contain the following information:

The locality
Date
Name of collector

This label should be on the pin below the specimen, leaving about the same amount of space between the label and insect as there is between the insect and the head of the pin.

For specimens in vials the same information should be written with India ink on a small bond-paper label, which is placed inside the vial.

Name of Species (Genus and Species)

The scientific name of the insect should appear on a label which lies against the floor of the box or case. It should be pinned in place by the pin which carries the insect. When more than one specimen of the same species is to be mounted, each insect may be labeled or only the first.

Order and Family Label

The order and family labels are placed on the bottom of the case ahead of the insects belonging to that group.

Care of the Insect Collection

After a collection of pinned insects has been made it must be examined three or four times a year for museum pests. Their presence is readily detected by sawdust-like debris around the bases of some of the pins. These pests can be killed by pouring into the box a tablespoon of carbon bisulphide, benzine, or carbon tetrachloride and keeping the box tightly closed. Since carbon bisulphide is highly inflammable, extreme care should be taken to keep all fires away. Ordinary moth balls will help keep the collection free from museum pests, but will not kill them, nor drive them away once they have become established. A heated pin can be thrust head-first into the moth ball and when cooled it can be pinned in one corner of the box. Care must be taken not to let the moth ball come loose and roll around in the box.

ECHINODERMATA

The Collection of Echinoderms

THE Echinodermata including the starfish, the sea-urchin and sea-cucumbers, the crinoids, etc. are all marine and are to be secured for the most part by hand picking at low tide or by dredging.

ASTEROIDEA

Adults are frequently uncovered by low tides and can be torn from the rocks or other places of lodgment at this time. They are also sometimes secured in large numbers in dredging operations. Preservation should not be carried on in very large or deep containers, as the pressure will in this case flatten and distort the specimens on the bottom. The early embryological stages of starfish are obtained by artificially fertilizing the ripe eggs in sea water in the laboratory. It is not easy, however, to keep the developing embryos alive. At the proper season a tow net drawn through the eel-grass just below the low tide water line will be almost certain to result in the capture of large numbers of the free-swimming larval stages. A few days later the recently metamorphosed young starfish may be gathered from the same vegetation to which they will now be found to be clinging.

OPHIUROIDEA

Brittle-stars may be picked up between tides by searching among the sea weed, under rocks and in other hidden situations. They may also be secured by dredging, especially on mud bottoms, while some species may be dug out of the sand at low tide. Some collectors preserve by drying. The chief difficulty with any method of preserving brittle-stars results from their tendency to fragment. This difficulty may be overcome to a considerable extent in specimens that are to be dried by soaking them for several days in a solution consisting of one-half fifty per cent alcohol and one-half pure glycerine. When the specimens are now removed from this solution and allowed to dry they will be found to possess sufficient elasticity to allow handling. They must be dried with care, for as a result of the presence of the moisture in the tissues they are liable to attack from saprophytic fungi.

ECHINOIDEA

Adult *sea-urchins* will be found near shore upon rocks which are partially or wholly exposed during low tides. In many places along the Atlantic coast they are very plentiful and can be collected easily. When large quantities are wanted they are usually secured by means of a dredge dragged along the bottom behind a motor boat. The locating of a good sea-urchin "ground" is not always easy, but, when found, as many as five hundred specimens of various sizes will sometimes come up with a single haul of a large dredge. Some species do not seem to occur in large numbers, however, and these forms must be hand-picked from the rocks at low tide.

HOLOTHURIOIDEA

Sea-cucumbers may be collected by hand between tides, or they may be dredged from sandy or muddy ocean bottoms. The method used depends upon the habits of the species to be secured. Once secured the animals must be handled with some care. They cannot be placed directly in ten per cent formalin, the procedure followed with starfish and sea-urchins, for when sea-cucumbers are powerfully stimulated, as for example, when placed directly into preservative, the powerful contractions of the body wall may result in rupture of the body, the whole crown of tentacles may be thrown off and even a portion of the viscera may be ejected, to the utter ruin of the specimen for laboratory purposes. One method of avoiding this trouble is to grasp the living animal immediately behind the tentacles with strong forceps and plunge it into boiling water or acetic acid. If the action is rapid the tentacles will remain partially expanded and the body walls intact. Another method is to stupefy the animals by placing them while alive in sea water to which chloretone or magnesium sulphate has been added. Or the anesthetic may be added slowly to the sea water after the animals have had time to expand in it. The effect of the anesthetic will be to decrease the sensitivity of the animals to a point where they can be handled and killed in extended condition. If left too long in the presence of the anesthetic they will die and macerate. An hour or possibly two hours should be sufficient. Once killed, the specimens can be preserved in ten per cent formalin and the same fluid should be injected into the body cavity.

Several species of sea-cucumber in the South Sea Islands and the China coast are collected, dried and baled to form the "bêche-de-mer," an item which assumes some importance both as an article of diet and of trade.

CRINOIDEA

The common crinoid *Antedon* Freminville or sea-feather is taken with plankton in various tropical seas, most of those used in American schools coming from the Mediterranean Sea. Great care must be used in handling the specimens as they are readily broken. The sea-feathers are commonly preserved in alcohol, but may be dried successfully if first placed in glycerine alcohol solution for a few days. The stalked crinoids are secured by dredging and since they are found only at considerable depths they are not readily obtainable.

The Preservation of Echinoderms

The hard-shelled echinoderms preserve readily in formalin solution which should be changed at least once before they are stored. The sea-cucumbers must be anesthetized if the tentacles are to show and the body to be in any way expanded. The habit upon the part of certain sea-cucumbers of breaking in two or evaginating their viscera when handled, offers an additional reason for anesthetization in this group. Among the brittle-stars some species are so fragile that they can scarcely be handled at all without breakage. Others, including the serpent-stars, do not break so readily. Our method with these species is first to soak them in a 10 per cent formalin 10 per cent glycerine solution and then after ten days dry them in the sun. Specimens thus treated will withstand considerable careless handling. The sea-urchins can be preserved in formalin or alcohol, preferably the latter. In either case the membrane about the mouth should be punctured in one or two places and the specimens then placed in a tray and the body fluids allowed to drain out. When thoroughly drained they may be placed in an alcohol-formalin solution. The sand dollars yield to formol-glycerine treatment as described for the serpent-stars, but different species vary widely in their friability. It is best usually to store these specimens in the dry form. Of the crinoids we know little except that Antedon is exceedingly liable to breakage and is best preserved in alcohol.

The Naples bulletin suggested the following methods of preparing museum specimens of echinoderms:

ASTEROIDEA

To prepare the starfishes with the ambulacral feet in a state of distention, allow them to die in alcohol of twenty to thirty per cent, being placed in the vessel ambulacra uppermost.

OPHIUROIDEA

Most of these may be allowed to die in fresh water because they thus remain distended and entire. Certain small forms can be fixed directly in weak alcohol, shaking the vessel violently to hasten death. Those having soft bodies are hardened in chromic acid of one-half per cent. *Ophiopsila* breaks in pieces of itself in fresh water, and is therefore killed in absolute alcohol.

ECHINOIDEA

To prepare the sea-urchins with the ambulacral feet well distended they are placed in just enough sea water to cover them and an equal volume of chrom-acetic mixture No. 2 is poured into the jar. They must be transferred at once to alcohol, so as not to give time for the acid to corrode the calcium carbonate of the animal. If desirable to preserve the soft parts of the animal for anatomical purposes, or even for form only, two small holes must be made in the shell opposite each other to discharge all the fluid contained therein. After immersion in alcohol one must see that the liquid fills the internal cavity and, on changing the animal to stronger alcohol, that the liquid contained within the shell is changed also.

If dry specimens of Echinoidea are wished, discharge the water within the shells and let the animals lie in ordinary 70 per cent alcohol for one or two days before placing them in the wind and sun to dry. Starfish also make better dried specimens if they are killed in 70 per cent alcohol and allowed to remain there two or three days before they are dried.

HOLOTHURIOIDEA

The sea-cucumbers require more care than other echinoderms, because they have soft and very contractile bodies and all are furnished with tentacles which contract or retire within the body on contact with a reagent. Some species, furthermore, soon after they are immersed in the fixing fluid expel their viscera and become valueless as specimens—a thing which may happen in the sea water also, if that is changed too suddenly. All these inconveniences are avoided by treating the animals by the methods here described.

First of all, as with other animals which must become expanded, they are placed in clear sea water. Those species which are killed in acids should be allowed to remain in them only just long enough to cause death, so that the calcareous cutaneous spicules be not injured. Large specimens of *Holothuria* and *Stichopus* as soon as the tentacles are fully distended shou'd be seized with two fingers, or with a pair of forceps, a little below the tentacles, lifted from the sea water and the anterior portion only immersed in a rather deep vessel containing concentrated acetic acid. At the same time another person should inject some 90 per cent alcohol into the animal through the anal aperture with a syringe, taking care not to exert too great pressure, lest the body be distended too much. Before the holothurian is quite dead it should be immersed in 70 per cent alcohol, and the anal orifice should be closed with a small cork to prevent the escape of the liquid and the consequent flattening of the body. The injection should be repeated at each successive renewal of the alcohol. With certain species the operations must be performed with much caution, because the skin is easily injured.

Thyone is strangled without using much force, wholly immersed in acetic acid, and then removed to alcohol as soon as dead. If the individuals are very small, the pressure on the neck should be made with forceps instead of the fingers.

Cucumaria plancii is treated like the large Holothurians, except that the injection of alcohol is made through the mouth, taking care to keep the tentacles extended, and it is not necessary to close the opening with a cork. The other species of *Cucumaria* are killed in the same manner. It is not necessary to inject the small specimens.

The large *Synapta*, in the preparation of which much difficulty has been experienced on account of its tendency to break to pieces, is killed by immersion in a tube containing sea water and ether in equal parts. In this mixture the animals usually die completely extended. When dead and well extended, they are washed in fresh water and put into weak alcohol. The transfer to 70 per cent alcohol should be very gradual, to avoid contraction. Chloroform may be used instead of ether. The hardening may also be done by putting two or three cc. of one per cent chromic acid into the water in which they have been washed. After a few seconds remove to weak alcohol.

CRINOIDEA

Antedon rosacea is put directly into 70 per cent alcohol, but *A. phalangium*, on account of its tendency to break in pieces of itself, must be killed in that of 90 per cent. Shake the vessel violently to hasten death and prevent the animals from breaking off their arms. When in doubt about the species use 90 per cent alcohol.

The larval forms, or pentacrinoids, are narcotized with chloral hydrate of 0.1 of one per cent—a process requiring two to four hours. If they are then hardened in alcohol, they will remain with the arms perfectly extended. The more advanced stages are best killed with saturated sublimate, where, however, they should remain only a few moments to avoid injury to the membranes.

MOLLUSCA

NOTWITHSTANDING the fact that there are many species of land snails and slugs, the molluscs are preeminently aquatic in habit, the fresh-water mussels being typical of our streams and smaller lakes, while an almost indescribable variety of snails, clams and other types of molluscs inhabit the ocean in incalculable numbers.

The Collection of Molluscs

The collection of terrestrial and fresh-water snails is largely a matter of learning their habits so that the animals may be located and collected by hand. The land snails will be found in moist woods, under leaves or decaying logs, or in damp rainy weather they may be found crawling about on the ground, logs or even on the tree trunks. The slugs are largely nocturnal in habit and may, when numerous, be collected at this time with a flashlight. During the day they lie hidden in some damp dark crevice or nook. The fresh-water pulmonate snails such as *Physa* and *Lymnaea* may be swept in quantity from the aquatic vegetation with a dip net; or if they are bottom gill-breathing forms like the species *Pleurocera* Rafinesque and *Vivipara* Lamarck they can be picked or raked from the bottom. Nearly all the fresh-water snails avoid the deeper water, occurring for the most part in the shallows or along the margins of the deeper lakes, ponds and rivers.

The marine snails are also chiefly shallow water forms, most species having heavy shells and many of them burrowing in the sand of the littoral zone. Certain of the marine gastropods, e.g. the nudibranchs, molluscs known as the sea-pigeons and the sea-butterflies possess no shells, however, and are able to swim freely in the water by means of flappings of the modified mantle. These forms at times congregate in great schools when they may be netted in quantity. The cephalopod molluscs are trapped or taken by hand as the case may be.

Details of collecting some of the more common laboratory types of molluscs are given below.

Chitons can be pried off the rocks with ease and preserved directly in 10% formalin. Relaxing in hot water before preservation may tend to prevent curling although this is usually not serious with the larger species. Some of the smaller species, however, will curl up like a pill-bug when pried from their position on the rocks.

Dentalium Linnaeus can be collected by sifting the sand from the bars along a sandy ocean coast. Usually the proportion of empty shells to those containing animals is large, e.g. 10 to 20 empty shells to one that is inhabited. Thus the living animals are not usually abundant, one or two dozen being a good day's catch. A hundred or more empty shells may be secured in this same time. Preservation by dropping into 10% formalin is satisfactory.

Slugs may be collected during the daytime by searching for them in their damp retreats under leaves, stones, logs, etc., or at night when they will be found feeding on the vegetation. To preserve them they should first be anesthetized so that the stimulus of the preserving solution will not cause them to contract. This may be accomplished by first placing them in boiled water (but recently cooled) in a sealed bottle or jar. The animals will relax in the water under the anesthetic action of the absence of oxygen plus the presence of a high carbon dioxide content resultant from their own metabolic processes and after a time they may be removed from the water and hardened in formalin or alcohol without danger of contraction.

Limpets are readily collected at low tide when they may be pried from the rocks with a knife blade. The giant limpet, *Lottia* and the small keyhole limpet, *Fissurella* Bruguirere will not be found in the exposed positions occupied by the species of *Acmaea*. The hole in the shell of the keyhole limpet decreases its protection against desiccation and the animals are to be looked for farther out among the sea weed or on the under side of rocks where the exposure is diminished. The abalone or ear-shell, *Haliotis* Linnaeus, although it belongs to a different family, *Haliotidae*, is essentially a giant limpet in habit, being found in much the same situations as harbor the keyhole limpets. After a series of high tides abalones will be found at low tide on the rocks of the higher beach. When the tides are not so high they must be sought farther out. In collecting abalones the collector must observe some precaution. The shell of the submerged abalone may be lifted to a height of an inch or so from the surface. The collector who thinks to slip his fingers under the raised shell and lift the animal from its position is due for an unpleasant surprise, if indeed he does not get himself into serious trouble. Since a straight pull of several hundred to a thousand pounds or more is needed to drag a large abalone from the rock surface a man must be unusually strong if he is to prevent the clamping down of the shell and the crushing of his fingers. It may even be that his life will be endangered, for animals as large as coyotes have been caught and held by abalones until drowned by the incoming tide. With a short flat iron bar, e.g. a tire iron, the collector can pry the abalones off without danger, for pressure applied at one side of the shell removes the animal with much less effort than is required by a straight pull applied to both sides. There are times following extended heavy on-shore winds when abalones are thrown upon the beach in large numbers and at such times along the southern coast of California the truck farmers gather the fleshy parts in quantity to be used as fertilizer on their garden patches.

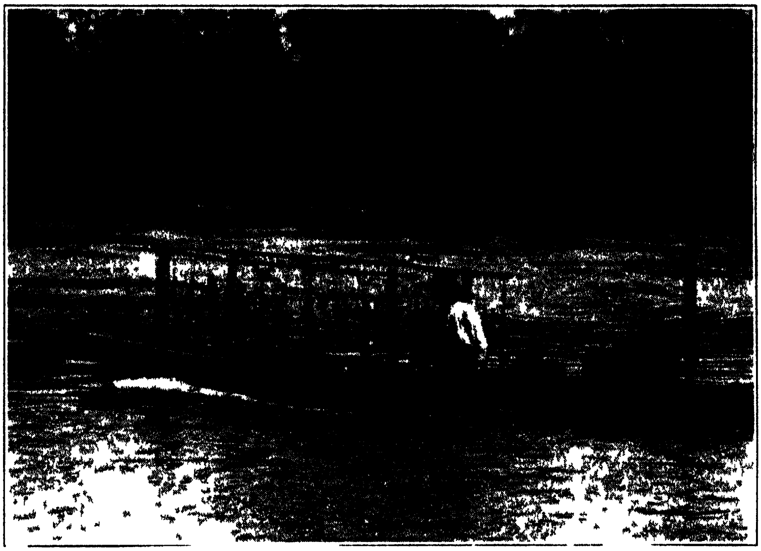
Mytilus Linnaeus, the sea mussel, may be hand-collected by tearing the animals from the rocks and other objects to which they are attached by the strong byssus.

For commercial purposes *oysters* are collected with dredges, oyster tongs, etc. The laboratory supply house secures live oysters in the shell from the larger fish dealers. Oysters may live for weeks and months outside the water without apparent deterioration with the shells closed so tightly as to prevent evaporation. Such oysters will preserve their vitality for a long period providing they are kept at a fairly low temperature. Oysters may be preserved in 10 per cent formalin by merely dropping them into the solution, as enough of the solution to kill and preserve the specimen will seep into the shell between the closed shell margins.

Adult *Mya* Linnaeus may be dug or dredged from the sandy or muddy bottoms in which they live. Laboratory specimens should be handled with care to prevent breaking the shell. The living clams can be killed by placing them in warm sea water. Once dead they should be preserved in formalin. Killing the clams in warm water results in their dying in extended condition. Specimens killed by dropping into formalin will so contract as to interfere seriously with examination and dissection.

Teredo Linnaeus can be collected only by splitting the animals out of infected pieces of wood, but it is not easy to secure complete specimens. The animals fit snugly into their burrows, which are long and tortuous, sometimes doubling back to form a U, or a V, or even a spiral and usually the animals will be broken in the process of drawing them from their holes. Small pieces of infected wood may be immersed for a few seconds in hot water to kill the animals, when they can be secured in somewhat better condition. At the best, however, the task of securing perfect specimens is a tedious one.

Lampsilis—In a shallow lake near Chicago the author, with two helpers, hand-collected ten thousand specimens of *Lampsilis luteola* Lamarck in one afternoon. The mussels did not cover the bottom by any means, but were abundant enough so that little searching was necessary. For years this same bed was visited in late summer with no apparent diminution in the number of mussels present. Then one summer a large sewer was built in the vicinity with the result that the shallow lake became dry except in the deeper parts. No mussels have been found in the lake since, although some water is still present over much of the former lake bottom. Commercial mussel collecting makes use of dredges, tongs, rakes and power boats insofar as the law will allow. Regulations licensing the collectors and limiting the size of boats and dredges are in effect in most states. The average collector drags a small dredge with a row boat. The dredge consists essentially of a horizontal bar or V from which hangs a fringe of long strings with three-tined hooks, the crow-foot hooks. As the hooks are dragged over the bottom the mussels are stimulated to close their shells and a certain percentage will clamp down onto one or more hooks. At intervals the dredge is hoisted into the boat and the mussels knocked or pulled off. Once taken from the water the mussels are left exposed to the sun and soon die, or they are placed in warm water to kill them. When dead the tissue slips easily out of the shell and the cleaned shells are stacked and later sold by the ton. The market price of the



An Arkansas "Clam Digger" with his boat and outfit.

Clams hanging to crow-foot hooks.

common river run of mussel shells may be placed at thirty-five to forty-five dollars a ton, though the price varies widely according to the quality of the shell. Straight yellow sand shell brought $130.00 per ton during the summer of 1927, but many grades of shells brought but $10.00 to $15.00 per ton. The total catch of mussel shell in the United States in 1922 was 29,007 tons valued at $1,268,740. A minimum size limit on mussels of 2 inches to 3 inches is set by most states. Only the enforcement of the laws protecting the mussel bed from too intensive collecting, combined with the establishment of measures for preserving the breeding beds from pollution, will save the industries which depend upon mussel shell for their existence, while methods of artificial propagation being developed by the United States Bureau of Fisheries are due to play an important part in the protection and intensive production of our mussel beds.

Scallops are collected for commercial purposes by dredging, the season beginning with September and lasting till late winter. On account of the peculiar habits of the scallop the catch in any single locality is always uncertain.

The native fishermen who catch *Nautilus* Linnaeus for food and because there is some market for the shells, do so by lowering traps to the bottom in waters known to be inhabited by the animals. The traps which are much like our own lobster traps are baited with some soft food, e.g. fish, the entrails of a cat or dog, etc. Occasionally a single nautilus is found floating at the surface. Such individuals are certainly out of their normal habitat and in all probability they are dying. The finding of such specimens led at one time to the erroneous conclusion that *Nautilus* like *Argonauta* is a surface floating form.

Octopi can be taken in baited crab traps, or they may be speared at night by the aid of an acetylene light as they hide in the rock crevices. The tendency of these animals to make their homes in hidden places beneath the rocks plays to the advantage of the fishermen, who lower pots to the bottom on long lines. These pots are 8 to 14 inches in diameter and 18 to 30 inches long. Each pot has a small hole in one end and without being baited will attract the octopi, which crawl into the pot, sometimes in numbers. Small octopi may be collected in some numbers by hand at low tide from their hiding places under the rocks and there are times when they may be strewn upon the beach by severe storms.

The Preservation of Molluscs

The *phylum Mollusca* is divided into several large groups, each group requiring a somewhat different method of preparation for the laboratory. The *Pelecypoda* may if desired be preserved by dropping directly into 10 per cent formalin, but it is desirable that bivalves such as the fresh-water mussels be placed in warm water until the valves open. A small peg of wood can then be inserted between the valves and the animal dropped into the formalin. The valves are thus already open when the mussels are given to the class and much time is saved for student and teacher. This same method may be followed with oysters, quahogs and all the tightly closing bivalves. *Teredo,* the ship worm, is preserved readily in formalin once it is removed from the wood. The chitons and snails may be preserved directly in formalin, but it is desirable that snails for dissection be anesthetized in warm water with magnesium sulphate to cause them to expand. Thus killed they make excellent preparations. The slugs may be treated in similar manner. Squids and octopi are usually preserved directly in formalin if desired for class work only.

PELECYPODA

For museum preparations pelecypods may be prepared with the valves open by narcotizing them in alcoholized sea water, where they should remain from six to twelve hours or even longer, according to the species. The siphonate forms should not be transferred to alcohol before they are thoroughly stupefied, otherwise the siphons will contract. After the animals have been narcotized, it is an excellent plan to place them in chromic acid of 1 per cent for about half an hour. They are very likely to open their shells wider after they have been placed in the chromic acid. When chromic acid is not used, place a bit of wood between the valves to keep them apart when first put into alcohol. Cocaine may be used instead of alcoholized sea water for narcotizing the animals, this method making it possible to preserve the specimens in a more extended condition, although its use is not as necessary for lamellibranchs as for gastropods, as described below.

Lima, which has a large number of tentacular filaments around the edge of the mantle that break off if alcoholized sea water be used, is killed with chromic acid one-fourth of 1 per cent. Large specimens, however, yield better results if treated with the copper sulphate solution first.

SCAPHOPODA

Dentalium is narcotized with chloral hydrate 0.2 of 1 per cent, in which it remains from ten to twelve hours and is then put into 70 per cent alcohol.

GASTROPODA

The use of cocaine for narcotizing all species of gastropods is strongly recommended. The solution consists of 2 grams of cocaine hydrochloride dissolved in 100 c.c. of 50 per cent alcohol. Place the animals in the least practicable amount of water. Drop in a few drops of the cocaine solution, after two hours add a few more and continue the operation until the animals are thoroughly insensible. The action is much slower in winter than in summer. To avoid the contraction into the shell, which is apt to take place with prosobranchs having a spiral shell, even when the narcotizing has seemed to be complete, draw the operculum as far out as possible with a pair of pincers and bind it to the shell.

If cocaine is not available, the gastropods may be fixed in fairly complete extension by dropping 70 per cent alcohol little by little into the sea water until the animals no longer respond to any stimulus, an operation which often lasts two or three days. Then they are killed by rapidly pouring concentrated acetic acid over them and transferred to weak alcohol at once. If one desires to get perfect specimens he must treat several at once, because out of every lot some are sure to remain more or less contracted. Species which, when treated in the manner just described, remain entirely contracted, may be treated by letting them remain for some days in a mixture of sea water and fresh water in equal parts and afterwards killing them with acetic acid.

OPISTHOBRANCHIATA

The *bullas* are slowly narcotized in the mixture of sea water and fresh water in equal parts or in alcoholized sea water and allowed to remain therein until thoroughly insensible. They are killed with concentrated acetic acid and transferred at once to alcohol.

The sea slugs such as *Aplysia* may be fixed in 1 per cent chromic acid, where they are allowed to remain from fifteen to sixty minutes, according to their size; or they may be narcotized in chloral hydrate solution 1 per cent (which may take twelve hours), killed with concentrated acetic acid, transferred at once to chromic acid 1 per cent, after half an hour put into 50 per cent alcohol and so on.

The *Pteropoda* are placed in a little water and the two wings allowed to expand, when saturated sublimate solution is poured over them. After a few minutes they are washed, placed in weak alcohol and so on up to 70% alcohol. *Criseis acicula* is well prepared by killing it with alcoholized sea water.

CEPHALOPODA

These animals furnish the best preparations when they are immersed in the preserving fluid while still alive. If they have been dead for some time when received, they can be made to regain their shape in part by allowing them to lie in sea water for about an hour. Then they had better be hardened in 1 per cent chromic acid for fifteen to sixty minutes, according to their size, after which they must be transferred to alcohol.

Octopods, if small, may be narcotized in chloral hydrate of 0.2 of 1 per cent and then immersed in alcohol, where they sometimes contract, twisting the arms about the body, but after the animals are dead it is easy to stretch the arms and arrange them in a natural position. The larger animals (of a length of 15 cm. or more) should be killed in 1 per cent chromic acid, where they are usually kept half an hour, although very large ones may remain in the solution two hours. After washing them in fresh water, transfer to 70 per cent alcohol, taking care to change the latter several times.

Squids and *cuttlefish* may be put at once into 70 per cent alcohol, taking care, before they are quite dead, to pull out the two tentacular arms which usually have contracted. The small species should be narcotized in chloral hydrate 0.2 of 1 per cent or in alcoholized sea water before they are put into the alcohol. Inject some alcohol through the ventral wall into the visceral region of the larger specimens with a hand syringe and fine-gauge needle to insure internal preservation.

The transparent pelagic forms (*Loligopsis, Verania*) are put first into Kleinenberg's solution and after an hour are transferred to weak alcohol, from which they are gradually changed to 70 per cent. The forms which are contained in a common gelatinous substance are fixed with chromic acid of one-half of 1 per cent and then transferred to 50 per cent alcohol, where they remain permanently.

CHORDATA*

The Collection of Chordates

The lower Chordates best known to the laboratory are *Dolichoglossus* Spengel (*Balanoglossus* Delle Chiaje), the *Tunicata* (sea-squirts and ascidians) and *Amphioxus*. Of these forms *Dolichoglossus* and *Amphioxus* are burrowers and are to be secured by digging them out of the sand. *Dolichoglossus* is a delicate form and each individual must be separated from the sand by floating it out in water to prevent breakage. *Amphioxus* is sturdy enough to permit of ready handling.

Amphioxus is widely distributed. *Branchiostoma lanceolatum* occurs on our eastern coast from Chesapeake Bay southward, and *Branchiostoma californiense* occurs on the Pacific coast.

During the month of March, 1926, the writer collected several thousand Amphioxus on the Gulf Coast of Florida. The specimens taken were found in the sand on the landward side of sand bars and were collected at low tide. The presence of the animals is indicated by small holes in the sand, the Amphioxus lying in the burrows near the subterranean water line.

Collecting was carried on as follows: A spadeful of sand in which Amphioxus were believed to be present was dumped into a sieve made of reinforced copper wire tacked onto a square frame which was used to sift the Amphioxus from the sand. Three to six or eight animals was the usual catch for one shovelful of sand. After shaking most of the sand through the sieve the remaining material containing the Amphioxus was dumped onto a square of black oilcloth where the animals flopped about like miniature eels or burrowed beneath shells and other debris.

In March, 1928, the same sand bars that yielded so prolifically in 1926 were again visited and but 6 or 8 specimens were found.

In California, *Branchiostoma californiense* is sometimes collected by dredging, some specimens coming from depths of as much as twenty fathoms. This species may also be collected along the sandy shores at the time of low tide.

Tunicata are either sessile or pelagic and may therefore be taken by hand as they grow attached to the vegetation in the shallow water or, in the case of such forms as *Salpa* Forskal, can be netted as they float free at or near the water's surface. Such forms as *Molgula* Forbes abound in protected weed-grown estuaries, while *Salpa* drifts with the currents, sometimes appearing in the bays in great swarms.

The Preservation of Chordates

The *Chordata*, including *Balanoglossus*, *Ciona*, *Molgula* and *Salpa* may be preserved in formalin, usually being placed in the solution directly as collected.

CEPHALOCHORDATA

Amphioxus is best fixed in Bouin's fluid. Fix for 24 hours, up-grade through 35% and 50% to 70% alcohol and store in the latter. If the animals are not wanted for microscopic structure but only for gross study or demonstration specimens, it is better to narcotize them with chloral hydrate and fix in ten per cent formalin.

ENTEROPNEUSTA

Balanoglossus may be killed with the Kleinenberg solution or in chromic acid of one-half of 1 per cent, but the former is much better. When narcotized in alcoholized sea water the animals remain well distended and straight. The tornaria larva is killed with the sulphate of copper and sublimate mixture. It is well preserved also with saturated sublimate or with the chrom-osmic mixture.

---

*The term "*Chordata*" is used here in a rather loose sense to apply only to the *Acraniata*, although we realize that strictly the vertebrates are also chordates. The vertebrates are treated separately in the following sections.

TUNICATA

Ciona intestinalis is killed slowly by putting into the water in which the animals have expanded a few drops of chrom-acetic mixture No. 2. When the animal has died, which happens in about half an hour, it is to be taken by the anterior orifice, to avoid the discharge of the water within, and put into 1 per cent chromic acid, injecting the same fixative into the body cavity. The animals should then be run up in the alcohol series, taking care to handle them by the anterior orifice as before.

Molgula, Polycarpa, Rhopalea and *Chevreulius (Rhodosoma)* must remain for twelve hours in chloral hydrate of 0.1 of 1 per cent. Then kill them in chrom-acetic No. 2 and transfer at once to 1 per cent chromic acid for a little time to harden. Menthol crystals placed on the surface of sea water in which *Molgula* is expanding will kill the specimens in the extended position.

Cynthia and *Styela* are narcotized in 0.2 of 1 per cent chloral hydrate for twenty-four hours and then treated like the above mentioned genera. *Cynthia papillosa,* however, sometimes contracts greatly when immersed in chloral hydrate of 0.2 of 1 per cent. When it does, put it back into running sea water to expand again and then try treating it with 0.1 of 1 per cent chloral hydrate.

Ascidiae compositae.—The gelatinous forms—for example, *Botryllus, Polycyclus, Circinalium* and *Fragarium*, are allowed to lie in chloral hydrate of 0.1 of 1 per cent for some time and then are killed by pouring hot saturated sublimate over them. Immediately afterwards they are transferred to chromic acid of one-half of 1 per cent, where they are left for a half hour before they are washed and transferred to alcohol.

The *Salpidae* include animals of very diverse consistency, from slimy to cartilaginous. Furthermore, certain species, although being relatively firm when young, become soft in the adult stages and are difficult to preserve. Sometimes the salpas, when immersed in the fixing fluid, contract greatly, closing the orifices and dying in this condition. This may be remedied by introducing a glass tube into one of the openings, which, by allowing the entrance of the liquid, causes the animal to resume its natural shape.

The species with hard bodies (*Salpa bicaudata* when solitary and young; *S. tilesi,* both chain and solitary forms; *S. zonaria,* both chain and solitary forms) are immersed in 10 per cent acetic acid in fresh water, where they remain for fifteen minutes. Then they are washed in fresh water for ten minutes and transferred gradually to alcohol, where it is necessary to float the larger forms by means of pieces of cork attached to them with threads so that the gelatinous sac will not flatten down upon the intestinal mass within. When treated in this manner, the animals remain very transparent, crystals of marine salts forming in the tissues much less than with the other liquids.

The forms of medium consistency (young chains and solitary individuals of *Salpa maxima* and *S. pinnata,* young chains of *S. bicaudata,* both the adult forms of *S. fusiformis* and *S. democratica-mucronata*) are placed in the chrom-acetic mixture No. 1 for ten minutes and then put directly into weak alcohol.

The very soft forms (large chains of *Salpa bicaudata* and *S. punctata,* both forms of *S. maxima, S. pinnata* and *S. virgola*) are immersed in the chrom-osmic mixture for from fifteen to sixty minutes, according to their size. Then they are washed in fresh water and transferred to weak alcohol.

Very large specimens of *Salpa maxima* flatten out of their own weight when put into weak alcohol. This may be obviated by blowing a little air into the cavity of the animal or by putting therein a tube of thin glass closed at both ends to act as a float. The tube or the air should be removed before the animal is entirely hardened.

One can easily inject the circulatory system of living salpas with Prussian blue by placing the point of a fine syringe in the slender canal of the heart and operating it with a very gentle pressure. After this the animals can be treated by the methods already detailed and the color will remain very well after they have been put into alcohol.

VERTEBRATA

It is comparatively easy to preserve and harden any vertebrate animals in a position that will be convenient for dissection or display, but it is practically impossible to take a specimen already hardened in a distorted position and prepare it so that it will look at all natural. With all of the larger vertebrates it is essential that the preserving fluid be introduced into the body cavity. This can be done by making a small slit through the ventral body wall before immersing the specimen in the preservative, or the preservative can be introduced via syringe and hypodermic needle. In all cases the preservation should be thorough from the beginning, for it is often exceedingly difficult to stop decomposition once it has begun. In animals as large as cats and dogs it is essential that the embalming method should be followed. By this method the preserving or embalming solution is injected into the blood system as soon as the animal is dead. If an embalming solution is used it must contain glycerine, carbolic acid and formalin. By filling the vascular system with this solution one can be sure of perfect preservation, for the solution does not remain in the arteries and veins, but penetrates the vascular walls to diffuse through the surrounding tissues. Embalmed specimens need never be immersed in fluid, but will keep indefinitely. For the convenience of the laboratory workers the specimens should be stored in a tight box to prevent too great drying out between periods.

CYCLOSTOMATA

The Collection of Cyclostomes

Lampreys are at times taken from fish to which they have attached themselves, but large collections can only be made in the spawning season in the spring when the sexes congregate in the smaller streams for breeding purposes. Here the adults can be netted without a great deal of difficulty. We have found the small brook lampreys very plentiful in trout streams in northern Michigan, where they gather for breeding during the first half of May. At this time as many as a dozen can be taken in one dip of a landing net, but at other seasons they are collected only in small numbers.

The *Ammocoetes* larvae burrow on the inner side of sand pits and must be dug up and separated from the sand as is described above for Amphioxus.

The Preservation of Cyclostomes

Lampreys quickly die when taken from the water and should be preserved as soon as possible after death. The body cavity should be injected with ten per cent formalin and the specimens then laid out to harden in formalin of the same strength. When the hardening process is complete the animals may be stored in fresh six to eight per cent formalin.

ELASMOBRANCHII

The Elasmobranch most used for dissection is the common dogfish shark, *Squalus acanthias,* of the Atlantic Coast. Other sharks are sometimes used, but for various reasons none of these have proved to be as satisfactory as *Squalus.* Skates and rays are also used to some extent, but only in small numbers in comparison with the thousands of *Squalus* that are dissected each year.

The Collection of Elasmobranchs

The collector who wishes to secure and preserve dogfish and other Elasmobranchs must do this work upon the sea-coast and, if he wishes to preserve more than a few specimens, he must have considerable equipment and laboratory space at his disposal. If only a few specimens are desired they can usually be caught by means of hand lines or purchased from the commercial fishermen, who often find considerable numbers of undersized dogfish and skates in their fish traps. To secure a large number of specimens during a single season, however, it is necessary to take advantage of the migratory movements of *Squalus,* which means the necessity of being prepared to contract in advance with the owners of large fish traps for the desired number of specimens and, what is most important, to have the facilities for receiving and handling on a moment's notice the hundreds or thousands of specimens per day during the comparatively short period when they are abundant. This usually means an established laboratory and a crew of five or six experienced men. In some localities where dogfish occur fairly plentifully throughout the season it is possible to handle them in smaller lots and spread the preparation work over a longer period. In general, however, specimens of this type can be collected and preserved economically only when they are handled in considerable quantities.

The Preservation and Injection of Elasmobranchs

Dogfish die very quickly after they have been taken from the water. The Elasmobranch liver will decompose very soon after death and preservation should be begun as promptly as possible after the specimens have been collected. Ten per cent formalin should be injected into the stomach through the mouth and the spiral valve should be filled with the same solution by injecting through the anus. In addition to this the body cavity should be filled with ten per cent formalin to prevent the decomposition of the liver. When this process has been completed the specimens must be laid out in ten per cent formalin and allowed to harden for three or four days, after which they may be transferred to storage tanks filled with fresh formalin of ten per cent strength. At no stage in the preservation should the specimens be allowed to pile up, as this forces the preservative out of the body cavity and alimentary canal.

When the blood vessels are to be filled with colored material, starch mass must be injected into the circulatory system before the animal is preserved. This mass hardens after injection, so that the arteries and veins may be easily dissected by the student. The formula for the starch mass is:—

Corn starch	1 lb.
Glycerine	100 cc.
Formalin	100 cc.
Water	400 cc.

Mix thoroughly and add color. Carmine is usually used for the red color, Berlin blue for the blue and chrome yellow for the yellow. Strain into glass jars. The starch mass must be thoroughly mixed each time before being used, so that it is free of lumps which might clog the cannulae.

To inject the arterial system of the dogfish proceed as follows: Cut through the tail just back of the last dorsal fin. This sections the vertebral column and exposes the haemal arch. The upper vessel in the haemal arch is the dorsal aorta. A short (½ inch) 16-gauge cannula is placed in the dorsal aorta and, by means of a hand syringe or pressure tank, yellow or red starch mass is injected anteriorly until the arterial system is entirely filled.

The hepatic portal vein and its branches are filled by slitting the ventral body wall, pulling out the spiral valve and injecting blue anteriorly into the vein which extends along the dorsal side of the spiral valve.

The systemic veins (anterior and posterior venae cavae) are filled by injecting anteriorly into the lateral veins which extend along both lateral body walls. This fills the huge posterior cardinal sinuses and requires a large amount of starch mass.

After injecting the blood vessels, the specimens are hardened and preserved as directed at the beginning of this section.

PISCES

The Collection of Fishes

The taking of fish is carried on largely with nets, although commercial hook and line fishing is a common practice in all of our waters. The catfish, which form an important source of food especially in the southern states, are caught on lines which are set on or near the bottom and baited with meat of some sort. Gulf fishermen, after the close of the mackerel season in March or April, catch large quantities of mutton fish, groupers, snappers, yellow-tails, king fish, etc., by fishing with hook and line along the coral reefs. Line trawls are extensively used by New England coast fishermen for deep-water fishing, while hand lines are common nearer shore. A line trawl consists of two parts, the ground line and the gangings, together with buoys, buoy lines and anchors. The ground line lies along the bottom and bears at intervals of about five feet the ganging lines to which the hooks are attached. The whole is buoyed to the surface by a buoy line. Again, certain of the larger fish, e.g. the sword fish of the New England coast, are captured by spearing or harpooning, while in this same region many other types of food fish are taken in traps, these traps sometimes covering an area of something like a city block.

Many types of nets and traps are used by the scientist who collects fish as well as commercial fisherman. Among these are the gill net, fyke net, purse seine, trawl net, pound net or fish trap, and other types of lesser importance.

The use of nets, particularly large seines, is prohibited in many states and the amateur collector is advised to consult his State Conservation Department before purchasing or using any of the larger nets, fish traps or set lines. Much local collecting of small fishes can, of course, be carried on by means of small seines and dip nets, which are usually permitted if their use does not seriously deplete the supply of young game fish.

The Preservation of Fishes

Practically all fish may be preserved in formalin, although specimens intended for the museum will keep in good condition longer if they are preserved in pure grain alcohol. Small fish such as minnows, darters, dace, etc., may be dropped alive into full-strength formalin. These specimens will die with fins and body extended and they may then be preserved in eight per cent formalin solution or in 85 per cent alcohol. Larger fish must be preserved by injecting preservative into the body cavity, as explained for Elasmobranchs.

The problem of preserving some of the beautiful natural colors of fishes is an extremely difficult one and so far no satisfactory preservative has been discovered. Some species (not many, unfortunately) will retain their natural colors fairly well in a solution of six per cent formalin in which a considerable amount of cane sugar has been dissolved. The necessary percentage of sugar must be determined by experimentation and, in any case, results are rather temporary.

AMPHIBIA

The Collection of Amphibians

Rana, Bufo, Necturus and *Cryptobranchus* are probably the most important laboratory amphibians, but many other forms can be collected in small numbers. The collection of amphibians is largely a matter of securing them one by one in the places and at the times when they are most plentiful; in meadows and about the borders of marshy lakes during the warmer part of the year. They may sometimes be found in small numbers in spring holes during the winter. In the Chicago region grass-frogs lay their eggs about April first. Toads congregate about ponds during the spawning season early in June, at which time large numbers of adults and eggs can be secured. Necturi are taken by means of hook and line, fish traps and nets in the late fall and early winter.

Some regions are much richer than others in amphibian life. In the Chicago region, for instance, toads and frogs are abundant, but salamanders are somewhat uncommon. On the other hand, we collected over fifteen species of salamanders during a single week in the Great Smoky Mountains of Tennessee, but found few frogs or toads in that region. The collector interested in Amphibians will do well to review the published data pertaining to his region and to learn as much as possible about the breeding seasons of the forms he wishes to collect.

Frog eggs may be collected in most localities for a period of only a week or ten days in the spring of the year. The eggs are frequently laid before the ice has entirely disappeared from the breeding ponds. The eggs of the grassfrog are laid in gelatinous clusters. They will usually be found attached to sticks or submerged grass in the shallow parts of ponds. To get the unsegmented eggs it is necessary to be on hand at the collecting ground early in the morning and fix the eggs in the field as soon as they are collected.

For gross study the eggs may be fixed in 8 per cent formalin while, for sectioning, Tellyesnicky's fluid is most satisfactory.

The Preservation and Injection of Amphibians

Frogs and toads are quickly killed by ether or by immersion in 80 per cent alcohol. After death the body cavity should be injected with 5 per cent formalin, the animal hardened in the same solution and then stored in fresh 5 per cent formalin.

To inject the arteries with color mass proceed as follows: Expose the heart by cutting open the pericardial cavity and cutting the pericardium which surrounds the heart. Inject red or yellow starch mass into the ventricle with an 18-gauge, 1 inch needle and a small hand syringe. Hold the heart in such a way that the starch will not back up into the auricle.

Necturus and *Cryptobranchus* may be killed by means of ether or alcohol, as directed for frogs. After the specimens are dead, 5 per cent formalin should be injected into the body cavity and the specimens then laid out in trays of the same solution to harden. After two days they may be transferred to permanent storage containers in fresh 5 per cent formalin.

To inject the circulatory system of *Necturus* with color mass proceed as follows: Open the body cavity by making a longitudinal slit through the ventral body wall about one-quarter of an inch from the mid-ventral line. Inject blue starch mass anteriorly into the postcava, inserting the needle into the posterior portion of this vessel. Inject until the heart fills and until both posterior cardinals are full.

The hepatic portal system may be injected by inserting the injecting needle into the intestinal branch of the hepatic portal vein. In making this injection be sure that the anterior abdominal vein also fills.

The arteries are filled by making two injections (one anteriorly and the other posteriorly) in the dorsal aorta. Make the incision in the anterior half of the aorta as it passes through the body cavity. Care must be exercised not to cut the posterior cardinals when the incision is being made in the aorta.

REPTILIA

The Collection of Reptiles

Reptiles, like amphibians, must usually be collected by hand and in small quantities, as it is the exception rather than the rule to find large numbers of reptiles gathered together within the limits of a small area. The exceptions are worth noting, however. Turtles are sometimes extremely abundant in certain ponds, where they may be captured by hand, with nets or in traps. They are usually most in evidence when the weather is warm and the sun bright, a rule which also applies to nearly all reptiles. In some of our less-frequented southern swamps alligators are still numerous and lizards of several species are abundant in some parts of our southwestern deserts. Some snakes den up for the winter in "communities" and, when the hibernating season is over, they can occasionally be captured in very large numbers when they emerge from their rocky retreats during the spring months. In general, however, the collector of reptiles must be content to capture his specimens one by one. Alligators and terrapins may be purchased from breeders, as both of these forms have commercial uses.

The Preservation and Injection of Reptiles

To preserve *turtles* proceed as follows: Kill the specimens by drowning (which requires several hours), or by injecting about 10 cc. of ether into the body cavity. As soon as the specimen is dead inject 10 per cent formalin into the body cavity, continuing the injection until the head is forced outward and the neck is distended with preservative. If the specimens are large the same preservative should also be injected into the muscles of the legs. They may then be placed in the storage solution of 8 per cent formalin.

To inject the arteries of turtles with color mass saw through the shell and remove the plastron to expose the heart. Tie off the auricles and inject red or yellow starch mass forward into the arteries by way of the ventricle until the intestinal arteries are well filled.

To inject the veins and hepatic portal system inject blue starch mass posteriorly into the left ventral abdominal vein. This will fill the renal portal system. Then fill the hepatic portal system by injecting anteriorly in the same incision.

To fill the anterior and posterior cardinals inject yellow starch mass anteriorly in one of the external jugulars; then insert the needle behind the incision and inject posteriorly until yellow begins to show up in the liver.

Snakes may be preserved by injecting 10 per cent formalin into the body cavity. If the specimen is a large one the preservative should also be injected under the skin of the back and into the muscles of the tail. Harden in 10 per cent formalin and store in formalin of 8 per cent strength.

To inject the arteries with color mass tie off the heart to separate ventricles and auricles and inject forward into the ventricle. The veins may be injected through the ventral abdominal vein.

Alligators are very difficult to preserve well because the skin is impervious to fixatives. Not only the body cavity, but the muscles of the head, back, tail and legs, must be injected with 10 per cent formalin. Harden for several days in the 10 per cent formalin and store in the same solution.

The injection of the circulatory system of the alligator is essentially the same as the method followed with turtles.

AVES

The use of birds for laboratory dissection is largely confined to sparrows, pigeons and the domestic chicken. Wild birds are collected for scientific purposes, but this work should be undertaken only by serious and experienced bird students who have a real need for the specimens taken. There are already so many excellent collections of bird skins in existence that there is no longer any excuse for the promiscuous collecting of our native birds. Most species are now rigidly protected by both state and federal laws and, aside from the taking of game birds in the open season, birds may be killed only by holders of special permits. To secure a scientific collecting permit it is necessary to apply to your state Department of Conservation and to the U. S. Biological Survey at Washington, D. C. Permits are issued only to persons who can prove some very good reason for the taking of bird specimens.

The Collection of Birds

For all-around bird collecting the best gun is a 12-gauge, double-barreled, breech-loading shotgun with barrels about 28 inches long. One barrel should be choked, the other cylinder cored and the latter should have fitted to it an auxiliary barrel for .32 caliber shells which latter should be loaded with No. 12 shot. With this auxiliary barrel one is prepared for the smallest specimens. A .22 caliber rifle or revolver is a good gun to have for shooting about the house or yard. In addition to the gun, one should be equipped with skinning scalpels, scissors, straight and curved, forceps short and long for stuffing and bone forceps. The preservative needed for the skins of birds is powdered arsenious acid (common arsenic) which may be used either pure or mixed with finely powdered alum in the proportion of two to one. Some persons prefer arsenical soap to the dry arsenic. The purpose of the arsenic treatment is to prevent the attack of museum pests but to make sure, the bills, feet and other unfeathered parts should be painted with an alcoholic solution of the oil of bitter almonds, oil of red cedar, camphor or other highly pungent substance.

Immediately after a bird is killed it should be picked up by the feet (never by the tail) and held head downward to allow the blood to drain from the mouth. Carefully remove blood clots which may adhere to the bill or feathers or to other parts of the body and apply corn meal to absorb what may remain. Clean sand may be substituted for the corn meal if necessary. Plug the holes made by the shot with cotton and plug also the throat and nostrils to prevent the escape of more blood and the stomach contents and juices. Before plugging the throat insert in the mouth a small quantity of corn meal to aid in absorption of the blood. A broken eye should be carefully dried and plugged, as the fluid from the eye once dried is particularly difficult to remove from the feathers. Pains thoroughly to clean the plumage at once will save a large amount of time later. In very warm weather before the throat is plugged the gullet should be swabbed with carbolic acid on a wad of cotton. The bird when cleaned and plugged should be carefully dropped head down into a paper cone of suitable size. The open end of the cone should be folded over the tail, care being taken not to break the latter and also to avoid doubling the head as will happen unless the bill is kept exactly in the middle of the cone. Wounded birds, if small, should be killed by squeezing the sides of the body which will produce death almost at once by suffocation. With large birds seize the legs and puncture the breast to the heart with a sharp scalpel.

The Preservation of Birds

As soon as the collector is back from the field the birds should be taken out of the cones and, if the weather is hot, their throats swabbed with a little carbolic acid and a fresh cotton plug inserted. It is best that the skinning should be attended to at once, although in cold weather this may be delayed several days if necessary.

To Skin the Bird: Relax the legs and wings by pulling and stretching; then make an incision through the skin only from the lower breast bone to the anus. Carefully separate the skin on one side from the subjacent parts until the thigh is exposed and the knee is reached. Taking this leg in one hand push the knee up onto the abdomen and loosen the skin around it until, with scissors or knife, the joint can be separated or cut through. Apply corn meal between the skin and the carcass to prevent adhesion and to keep the feathers clean and repeat with the other leg. Now loosen the skin at the base of the tail and cut through the vertebra at the last joint, taking care not to sever the bases of the tail quills. Loosen the skin from the rest of the body, peeling it forward in everted position with the raw side out, and when the wing is reached, loosen the skin about the base and cut the wing loose either at the body point or through the middle of the main wing bone. Draw the skin up over the neck until the skull is exposed, detach the delicate ear membrane from its cavity in the skull and loosen the skin, using the thumb nails, up to the eyes where extreme care must be exercised to avoid lacerating the eye ball when the nictitating membrane is cut. Scoop out the eyes and by making one cut on each side of the head through the small bone connecting the base of the lower jaw with the skull, another across the roof of the mouth behind the base of the upper mandible, a third between the jaws of the lower and a fourth (horizontally) through the skull behind the orbits and parallel to the roof of the mouth, you will have freed the skull from all the accompanying brain and muscle tissue. In making the first two cuts be careful not to injure the bone (zygoma) which connects the bases of upper and lower mandibles on each side. Clean out every particle of muscle and fat from the head to the very base of the bill. Skin the wing down to the wrist joint, detaching the roots of the larger feathers with the thumb or finger nails; remove the muscle from the bones, leaving all of the latter. Skin the legs to the lower thigh joint and remove all fat and muscle tissue. Remember throughout: (1) Do not stretch the skin, (2) do not soil the plumage, (3) use plenty of corn meal to keep the innner surface dry.

Once the skin is cleaned, apply the arsenic powder thickly to the raw surface, using a brush, a cloth or a rabbit's foot. Do not let the skin dry before applying the arsenic. Shake off the excess powder and turn the skin right side out. Now with long forceps push cotton up into neck and wing sockets, down into the legs and into the body until the skin is held plumply in position.

The skin should now be bound lightly with thread to close the opening while the skin is drying. After the skin has dried this binding may be removed. In case of large skins the opening may be sewed. Such a preparation is known as a study skin and should be accompanied by data regarding season taken, locality, age, sex, colors, etc. Bird skins should be kept in light-tight containers to prevent fading. Skins prepared as study skins can be mounted at any time by relaxing the skin.

Birds may also be preserved entire in alcohol or in formalin. Because a bird's skin is impervious to preservative, however, they can be prepared properly only by injecting the preservative into the body cavity and the larger muscles. Large specimens must have 10 per cent formalin injected into the crop, the body cavity, the legs and the breast muscles. Large birds can also be embalmed through the femoral artery and can then be kept for some time without storing in formalin.

The arterial system may be filled by injecting colored starch mass proximally into the femoral artery. The veins are not usually injected because they are very thin walled and therefore apt to rupture.

MAMMALIA

Specimens of small mammals may be preserved as skins, as skeletons and entire in alcohol or formalin. Mammals should be skinned as soon as possible after death, for they spoil much more quickly than birds. In hot weather the viscera should be removed at once following capture and the abdominal cavity filled with cotton, tow or leaves. This precaution is especially important with rats, mice, shrews and rabbits, or with any specimens which must be kept over night before skinning. When necessary the animal may be injected freely with dilute formalin or 75 per cent alcohol by means of a hypodermic syringe. The formalin solution should be saturated with salt.

The Collection of Mammals

Cats, rabbits, rats and most of the other mammals commonly used for laboratory study may be procured by purchase at all seasons. Wild animals must usually be trapped or shot. Small forms such as shrews, mice, moles, rats, etc., are easily collected by traps, the ordinary mouse and rat traps being quite satisfactory for this work. Inasmuch as this phase of scientific collecting is a large and specialized one, the reader must refer to the standard texts on taxidermy and to the special bulletins published by the U. S. Biological Survey and the larger natural history museums of the United States.

The Preservation of Animal Specimens

A—STUDY SKINS. If a gun has been used the bullet wounds will undoubtedly cause considerable bleeding. Carefully wipe off all blood stains by means of a small wad of cotton. If the animal cannot be skinned immediately, insert a small pack of cotton in the wound to prevent blood from spreading over the hair. If at all possible the skin should be removed from the animal within one to two hours after death. If one has along a small amount of salt he can always use it to good advantage in preserving the specimen.

Notes, Drawings, etc. We cannot here over-emphasize the necessity of taking full notes on all specimens intended for skins. Notes should, of course, have been taken in the field when the specimen was killed and should be supplemented by detailed notes taken in the laboratory or preparation room. Note the color of hair, color of eyes and various soft parts. The soft parts will lose their color when the skin is dry and if it is necessary to mount the skin it will be almost impossible to get the exact life-like coloration unless complete description has been made. It is recommended by some that a rough drawing should be made and colored either with oil or water colors to match the colors appearing in the fresh skin. Complete measurements are also necessary. After the head has been stretched out as far as it will go, one should measure from the tip of the nose to the root of the tail as a first measurement. Second, determine the length of the tail by measuring from the root of the tail, that is, the point where the tail joins the body, to the end of the vertebrae. Third, determine the length of the hind foot. This is done by bending the heel at a right angle and measuring from the outer extremity of the angle to the tip end of the longest toe, even including the toe nail. Unless the animal is very small it would be well to determine its height at the shoulders. To determine this one should measure from the sole of the foot to the top of the shoulder, the foot being in the position it would assume if the animal were standing erect. Penciled outlines of an animal are frequently valuable. These may be made

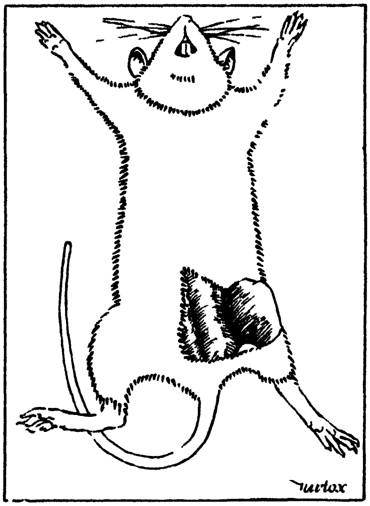


Fig. 1. Rat, showing first incision in belly skin, and knee joint in position for cutting.

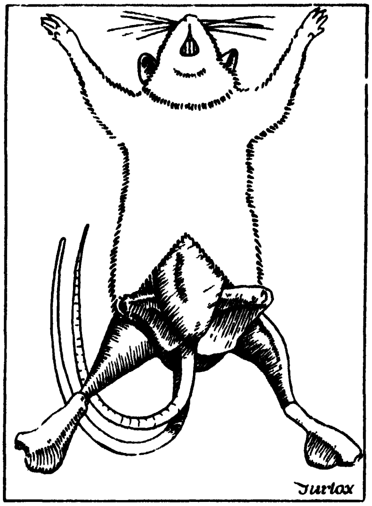


Fig. 2. Both knee joints having been severed, the skin is pulled down the legs.

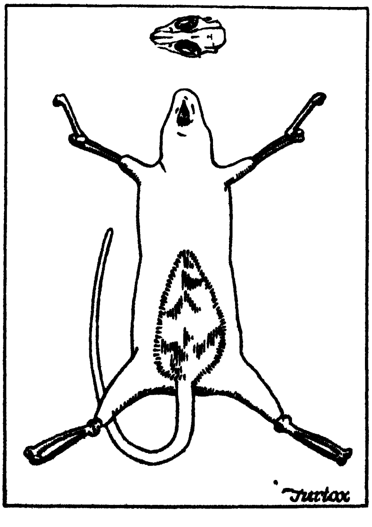


Fig. 3. Body has been completely removed from the skin. All bones having been fleshed, skull is detached.

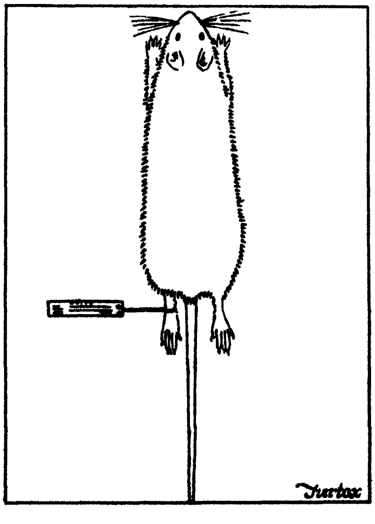


Fig. 4. Finished Museum Skin. Note extended limbs, natural body shape and label.

by placing the dead specimen upon a piece of paper and by tracing around it. At least these outlines are useful in securing proper proportions in case the skin is afterwards mounted.

Skinning the Specimen. We will now assume that a good specimen has been brought in for the purpose of preparing a study skin. Place the animal upon its back, preferably with its head to your right. By holding your sharp knife or scalpel with the edge up you may insert the point just through the skin of the belly at a point slightly more than halfway from the neck to the anus. It is not essential to have a very large opening for removing the body and the less cutting that is done, the less sewing there will be afterwards. By means of the thumbs and fingers carefully separate the skin on either side of the incision from the underlying structures, working as far around on the back of the specimen as possible. In most cases it will be easy to loosen the skin all the way around. Gradually work back toward the tail, cutting the flesh from the anus so that the skin, very shortly, can be stripped off of the tail. By means of the thumb and fingers gradually push back the skin from one of the hind limbs bringing the knee joint into full view. Figure 1 on the opposite page shows this step quite plainly. Cut the leg loose at the knee joint and then draw the skin down to the foot. Remove all of the flesh from the leg bones very carefully. Repeat the same process on the other hind limb (see figure 2) and then draw the body out through the opening, turning the skin inside out until the shoulders are reached. Cut the muscles binding the shoulders so that the front limbs are now loose. Remove all of the flesh from the bones of the front limbs clear down to the toes. It is not necessary to save the shoulder blade so this may be cut off at the joint between it and the humerus. Draw the skin very carefully over the neck of the animal continuing to turn it inside out as progress is made. The skin will come loose from the neck region quite easily, but some difficulty will be encountered when the head is reached. Much patience must be exercised here, otherwise the skin might be torn. When you come to the ears cut them off close to the skull. Bring the skin wrong side out down over the head until you come to the eyes. Work very slowly, keeping close to the edge of the bony orbit. Cut very carefully now so as not to injure the eyelids. Bring the skin on down to the end of the nose and cut through the cartilage close to the bone. Now cut down to where the upper lip joins the gums. Do not injure the lips by cutting them away at the edge of the hair. If the lips are fleshy they must be split open from the inside so that the flesh can be removed. If the ears have hair upon them they must be skinned up from the inside and turned wrong side out in order to separate the outside skin from the ear cartilage. Extreme care must be taken in this operation, especially if the ear is flabby.

The skin is now completely separated from the body and is turned inside out as in Figure 3. Cut any remaining muscles or fragments of muscles that may be attached to the skin and remove any fat. Preservatives will not penetrate through the skin to the epidermis quickly enough to save it from decomposition, especially if a thick layer of flesh has been left attached to the skin. The next step is to remove all of the muscle masses from the skull, this having previously been severed from the spinal column. The tongue and eyes are cut out and the brain is removed through the occipital opening of the skull by means of a brain spoon. If this instrument is not on hand one may use a flattened piece of wire for the same purpose. After the brain has been broken up thoroughly by the wire it can be shaken out through the opening. Running water sometimes aids in removing particles of brain. The skin and skull should now be washed carefully in separate containers. If any blood has reached the skin be careful to remove it in the water bath. Blood stains that have dried upon hair are often impossible to remove completely.

Poisoning the Skin. After the skin has been removed from the animal and properly cleaned the next important step is to sew up from the inside any holes that may have been made by the bullets in killing the animal or cuts which were made during the skinning process. A mixture of two-thirds powdered alum and one-third arsenic should be prepared. Rub this mixture on the inside of the skin until no more will cling. The alum preserves the skin, causing the hair to remain in good condition, and the arsenic poisons the skin against insects which so frequently attack skins that have not been prepared properly. In this operation wear rubber gloves; arsenic is poisonous and must not be handled with bare hands. Museum beetles (dermestids) attack and render worthless a museum skin within a very short time if it has not been poisoned properly. They attack first of all the feet, ears, head and tail and for this reason the arsenic-alum mixture must be applied to these parts very thoroughly. Arsenical soap is also used quite commonly in poisoning museum skins. This can be made up if one has the necessary ingredients, but if one has only a small number of skins to prepare it will probably pay him to purchase the soap from a supply house rather than go to the trouble himself. The soap should be painted on the skin, being careful, of course, to make a very thorough application to all parts. The soap will readily be absorbed by the skin, after which a mixture of fine salt and powdered alum in equal proportions should be applied. The alum-salt mixture is for preserving the skin and the arsenical soap for poisoning against insects. After the poisoning has been done properly the skin is ready to be turned hair side out. First, however, the leg bones should be wrapped with a little cotton or cotton cloth or some similar material, just enough to barely take the place of the flesh. At the same time, the filling takes the place of the flesh and also keeps the skin away from the bone, allowing both structures to dry properly. Use just enough material in the legs to give them a neat and natural appearance. If this is done the hair will assume a natural position, giving the skin a life-like appearance. The skin is now turned right side out over the filling of the legs and we are ready for further work.

Filling and Shaping the Body. By using the same filling material such as cotton, cotton cloth and the like, fill out the head, neck and body to about the natural size of the animal when alive. A "false body" may be prepared, conforming to the natural body in size, by wrapping cotton or cloth about a stiff wire which has previously been shaped properly. For the exact measurements you may need to refer to the outline you took before the animal was skinned, or to the various measurements which were entered in your notebook. Do not insert more than enough material to give the body its natural size, otherwise the skin will be stretched out of proportion. One matter that must be given consideration before we go further is the disposition to make of the skull. There are three methods of using the skull. The most universal custom is to replace it in the head and around it insert a little filling so as to care for the muscles, etc., that were cut away. One method is to place the skull in the center of the body with the filling; another is to tie the skull to the leg with the label. With this matter taken care of one may, with needle and thread, sew up the skin over the body filling so as to give a natural appearance. The sewing begins at the anterior end of the skin and is continued backward until the opening is completely closed.

Drying the Skin. The skin is now ready for drying. It should be laid out on a smooth surface with the front limbs projecting forward, close alongside the neck and head. The hind limbs should extend backward in an easy position. The tail, if it has been stuffed, should extend straight out behind. In specimens where the tail is too long and might be injured, it is well to curve it up between the legs on the belly surface. The finished skin having been shaped properly should be a true representation of the natural animal in so far as size, color and the general topography are concerned.

After the skin has been given a good shape a label should be attached to one of the hind legs. This point is most important because a specimen without a label is practically worthless.

B—SKELETONS. A really good skeletal preparation is characterized not only by clean, white bones, but also by a correct and natural arrangement (articulation) of the bones, and a type of mounting that is sturdy enough to withstand the handling by many classes of students. Take, for example, a cat skeleton. Assuming in the first place that a good cat is the real basis of a good cat skeleton, a specimen is selected that is large and perfectly formed and old enough so that the bones are well hardened and the teeth fully developed.

Fleshing. The fleshing of an animal is one of the most important operations in preparing a skeleton. Great care must always be taken not to cut through connecting ligaments which hold the bones in position. On the other hand, the flesh must be removed very thoroughly, for if too much muscle or flesh is left on the skeleton, maceration (decomposition) takes place too fast and in a very short time the bones will all become separated. First, the clavicles should be dissected out. These small bones, when present, can always be located between the coracoid process and the episternum, but often do not articulate with either of these points. (Clavicles that do articulate are called true clavicles and others that "float" in the muscle, as in the cat, are called false clavicles.) Next, dismember the four limbs and remove the skull by carefully cutting between the atlas and condyles. The next difficult part is to dissect out the hyoid or tongue bones. This group of nine bones, which lie in the muscles of the throat, should be dissected out intact. Now flesh the limbs, trunk, tail, skull, hyoid and clavicles. In working on the trunk take great care not to cut through the costal cartilages of the sternum. Pass a stiff piece of wire (12 or 13 gauge), 18 inches long, through the vertebrae, moving the wire back and forth to clean out the spinal cord. Next clean the skull, removing the brain with a brain spoon or similar instrument. The skeleton is now fleshed and ready for maceration.

Only fresh material should be used for making skeletons. Do not attempt to use specimens that have been preserved in formalin.

Maceration. After the fleshing is completed examine the bones carefully to be sure that all of the natural cartilages and ligaments have been left intact, as they are essential to the proper setting of the bones.

Place the bones in a stone or earthenware container of suitable size and cover with water. (Note—never use acids or chemicals of any kind!) Do not allow any foreign substance such as metal, wood, etc., to get into the maceration jar, as these will discolor the bones. The maceration jar should be kept in a warm room where an average temperature of about 78° F. can be maintained. At this temperature the maceration period is usually from six to eight days. Toward the end of this time the bones must be watched carefully, as the maceration of the flesh should be halted just in time to save the natural ligaments. When the flesh "jells" and sloughs off easily, remove the bones from the maceration bath and rinse under running water, going over them with a stiff brush to remove all remaining pieces of flesh. If the maceration has been timed correctly, the flesh should all wash off easily, yet the ligaments should remain. This is very important. To make sure that all maceration has ceased, place the bones in a weak solution of caustic soda (about ½ ounce sodium hydroxide to a gallon of water) for one to two hours. After removal, wash in running water.

If the natural ligaments remain, the small bones are held together, while if the natural ligaments are destroyed it will be necessary to wire all of the small bones together, which usually results in a very unsatisfactory skeleton.

Bleaching. After all flesh has been removed and the bones thoroughly washed they are held in their natural positions for drying by running a stiff pointed wire through the vertebrae, forcing the point of the wire well into the sacrum.

Having accomplished this, bend the vertebral column into its natural shape. While the skeleton is still wet, space the ribs equally by stringing them with thread. The limbs are dried in shape by pinning on a flat soft piece of wood or cork. After drying, should the bones still be greasy, place them in carbon tetrachloride for 6-8 hours. Then, on removal, plunge them into hot (not boiling) water for about 5 minutes, adding a little household ammonia, which will help to bring the grease to the surface. If it is desired to have a perfectly white skeleton, remove the bones from the water and place them in a 10 per cent solution of hydrogen peroxide for four hours. Hot chloride of lime is also a good bleaching agent but tends to destroy ligaments. Use it only on the large disarticulated preparations.

Mounting. Mounting is largely a matter of experience and skill. Anyone with a little mechanical ability can work out methods of mounting on stiff wires or (in the case of large skeletons) on metal rods and tubes. In general, it is wise to enclose small skeletons in glass cases, as this protects them from dust and careless handling.

C—PRESERVED AND EMBALMED SPECIMENS. Small mammals intended for internal dissection may be preserved in some liquid such as alcohol or formalin. The liquid preservative should be injected by means of a syringe and needle of suitable size into the body cavity, the brain, the muscles of the legs and neck and underneath the skin of the abdomen, back and thorax. The specimens may then be placed in the liquid preservative, where they should remain until needed for laboratory use. Alcohol of 70 per cent strength and formalin of 10 per cent strength are satisfactory. If there is a possibility that the specimen may be made up later into a study skin or skeleton, alcohol should be used, although it must be remembered that immersion in alcohol will change the colors of the pellages of many of the mammals.

The larger mammals may be preserved in liquid, but forms as large or larger than a cat are best preserved by the embalming method. This method requires freshly killed specimens, however, and it is well to remember that little time should elapse between the death of the animal and the embalming process.

As soon as the animal is dead, and while still limp, it should be laid out in such a way that it will be easy to dissect after the body has hardened. The legs should be well extended, the head thrown back so that the nose points forward and the mouth should be filled with a wad of absorbent cotton. It is well to lay the specimen on a board, dorsal side down, and tie the limbs and head in the desired positions.

The embalming fluid which we recommend is a modification of Keiler's fluid, originated for use in preserving human cadavers for anatomical study. The formula for this solution:

Carbolic acid (melted crystals)	2.5 parts
Formalin	1.5 parts
Glycerine	10.0 parts
Water	86.0 parts

The carbolic acid and formalin are the chief preserving agents. The formalin gives just the right firmness to the muscles while the carbolic acid preserves the color of the muscles and gives the specimen a "clean odor." The glycerine prevents the drying out of the specimen during the laboratory period.

The embalming can be done through the femoral or the carotid artery. Cats, dogs, monkeys, etc., are best embalmed through the femoral because this artery is large enough to take a good sized needle. Guinea pigs, rabbits and squirrels should be embalmed through the carotid, because the femoral artery is very small in these forms. An animal may be considered well embalmed when the muscles of the legs are firm and the abdomen tight. Such specimens should stand for about twenty-four hours before being used for dissection. They will keep for months if stored in a cool room and protected from drying.

After the embalmed specimens have been allowed to stand for several days they are ready for injection with color mass. Starch mass may be used (see page 50) or a gelatin mass may be employed. The latter is better in some respects, but is difficult to use as it must be handled warm.

The red starch should be injected caudally into the common carotid. Expose this by making a longitudinal cut parallel with the fibers of the sterno-mastoid muscle. Be careful not to cut the external jugular or the jugular commissure. Dissect the common carotid away from the internal jugular vein and tie it tightly anteriorly. Posteriorly tie a loose knot around the artery, make an incision in the vessel, insert needle caudally and pull knot tight. For the cat, inject about 33 cc. of red starch mass, remove needle and tie the artery.

The veins may be filled by injecting into the external jugular. From 30 to 50 cc. of blue starch mass should be used, the amount depending upon the size of the specimen.

The hepatic portal system must be injected through an intestinal branch of this vein. Cut through the lateral abdominal wall, pull out a coil of the intestine and inject into one of the larger intestinal branches. Use a 19-gauge needle for this injection. Tie off the vessel when the needle is removed, and sew up the incision in the body wall. Also sew up the incisions made through the skin of the leg and neck.

During the embalming process embalming fluid, mixed with blood, escapes into the body cavity. If the specimen is stored for some time this fluid gradually evaporates and may leave behind a dark bloody residue which coats the abdominal organs. This can be removed by rinsing the viscera with running water.

PRESERVATIVES

ON the following pages we have listed and discussed all of the basic killing and preserving solutions. There are countless other solutions developed and modified for certain special uses; to offer the formulae for all of these would be merely tedious. We have listed a considerable number of special preservatives, however, having selected the ones which we have found to be especially successful for certain designated purposes.

"The Ideal Preservative"

The ideal preservative of plants and animals is one that will retain the natural appearance of the specimen both externally and internally. There should be no distortion, the natural colors should be retained, and decomposition must be effectually prevented. Unfortunately, our present methods of preserving specimens frequently fall short of the ideal result and there is room for much experimental work upon the part of laboratory preparateurs and museum workers in the development of new and better procedures.

Specimens are preserved with a dual purpose in mind, first to retain the natural external appearance, and second to preserve the internal tissues and organs so that they will remain intact in their normal position and condition. It may be argued that natural preservation of the external appearance of specimens is not necessary when the material is to be used for dissection purposes, but it is nevertheless true that specimens which retain the appearance of the living animal, to a degree, are best for dissection purposes both because the natural appearance does away to a considerable extent with the feeling of repugnance which so often is associated with death and because of the fact that they are not twisted and distorted as is so frequently the case with carelessly prepared material. The smaller animal specimens, such as starfish, crayfish, insects, etc., for laboratory dissection work are usually preserved in fluid, while the larger forms such as cats, rabbits, large fish and others serve best when embalmed and stored dry, i.e., not in preserving fluid.

Formalin and **Alcohol** or some combination of these two substances make up the solutions most generally used in the preservation of the small animal forms. The formalin which one buys in its original container is frequently spoken of as 40% formaldehyde, which means a 40% solution of formaldehyde in water. However, the trade name **formalin** considers this solution to be 100% and most formulae which use the substance proceed on this assumption. Thus one preserves animal forms in 10% formalin solution, being at the same time a 4% solution of formaldehyde.

The alcohol which is so essential to biological work is unfortunately the same alcohol that enters so largely into the make-up of intoxicating liquors. The laws and rules of the prohibition enforcement department have, therefore, worked a hardship upon all biological institutions by making it exceedingly difficult to secure and use ethyl alcohol. At the time this is written, the securing of pure ethyl alcohol is a practical impossibility for all but the larger institutions.

BIOLOGICAL ALCOHOL. It is possible to secure a permit to buy alcohol, treated to make it unfit for drinking. Alcohol denatured as per formula No. 1 (see below) makes a satisfactory substitute for pure ethyl alcohol in most biological work, but this alcohol can be secured from the government bonding houses only by holders of government permits. Thus the workers who want only a few quarts of alcohol are ruled out because the bonding houses do not supply it in quantities smaller than five gallons.

The formulae of the specially denatured alcohols are changed rather frequently by the government and the collector who wishes accurate information on this subject must apply to his local prohibition director. At the time of this writing formulae SD No. 1 and SD No. 30 are as follows:

SD No. 1—
- 100 parts ethyl alcohol
- 5 parts approved methyl alcohol

SD No. 30—
- 100 parts ethyl alcohol
- 10 parts methyl alcohol

A special permit is required for the purchase of either of these alcohols.

FULLY DENATURED ALCOHOL. There are various formulae for so treating ethyl alcohol as to make it entirely unfit for internal consumption. Alcohol so treated is known to the trade as "fully denatured" and can be bought and sold like any other common commodity. Most of the fully denatured alcohols are poisoned with wood alcohol the same as Biological Alcohol, but in addition there has been added some substance such as gasoline, benzol or pyridine which causes the solution to turn milky when water is added to it. Rubbing alcohol usually contains some drug which acts as an emetic when the alcohol is swallowed.

Fully denatured alcohol is unsatisfactory for biological work because of the presence of these secondary denaturants which act in two ways to make the alcohol useless. First, the fact that the alcohol cannot be diluted makes it impossible to run material gradually from low to high grade solutions, a common procedure quite essential with most materials. Attempts to dilute the alcohol and use the solution in spite of the milky nature have failed, for a greasy white coating settles over the material, rendering it useless. The second serious objection to the use of fully denatured alcohol in biological work is that all the denaturing substances used have a drying effect upon tissues, with the result that the specimens become dry and brittle. This is especially serious with the arthropods, which, by the way, cannot be preserved satisfactorily in formalin, for the formalin and the denatured alcohol both result in the specimens becoming so brittle that the appendages break off in spite of the most careful handling.

WOOD ALCOHOL. Wood, or methyl alcohol, which is a product of the dry distillation of wood such as beech, is deadly poisonous if taken internally even in weak solution. This alcohol can be used as a substitute for ethyl alcohol in many biological procedures, but its poisonous properties are such as to prevent its wide use in laboratories. It is not safe even to breathe the fumes of wood alcohol from day to day. Wood alcohol is necessary to many commercial processes and to a few types of biological technique, such as that of staining blood smears, but in general its use in biological laboratories is not a wide one.

RUBBING ALCOHOL. The only usable alcohol freely available in small quantities to the general collector is the rubbing alcohol sold in pint and quart bottles at the drug stores. The formulae for rubbing alcohols vary somewhat, but the denaturant usually does not interfere with the preservation of such forms as insects and other small animals. Rubbing alcohol is already diluted to 70% and many specimens can be preserved directly in it. Unfortunately the retail price of this alcohol is usually too high to allow of its being used in any quantity.

FORMALIN SOLUTION (Standard)

Commercial formalin	10 c.c.
Water	90 c.c.

Formalin solutions of varying concentrations may be made up to meet special requirements. Some forms require a relatively strong solution, while others may be preserved to best advantage in a very weak solution. Many marine forms are preserved in solutions made up of sea water and formalin.

FORMALIN NEUTRAL SOLUTION. Neutralize the 100% formalin with an excess of either sodium carbonate or lithium carbonate and proceed as with the standard formalin solution. Much of the adverse effect of formalin solutions may be overcome by neutralizing the solutions in this way. Usually it is sufficient if an excess of the salt be kept as a sediment in the stock jar or barrel of pure 100% formalin. However, if specimens are to be kept permanently in formalin solution, the salt must be added to the containing jar, since the effect of light on formalin is to produce a deterioration with the production of free acids and an accompanying darkening in color of the solution, and eventually the specimen, unless the solution be changed frequently.

Alcohol Procedure. Since 70 per cent alcohol should be the final solution in which most permanent small animal specimens are to be kept, the general procedure of bringing the specimen to the final solution may be outlined here.

The procedure to be followed in introducing a specimen into 70% alcohol will depend largely upon the nature of the structure of the animal. Many animals may be dropped directly into the alcohol and left there with one or two changes until the solution no longer darkens. Specimens killed or temporarily preserved in formalin may often be transferred directly to 70% alcohol in the same way. Forms with soft, delicate bodies, i.e., the jellyfishes, must however, first be placed in 35 to 50% alcohol, left for at least six hours, then immersed in 60% for 6 hours and finally placed in 70%. Care should be used in changing the solution with such specimens or they will be damaged. The best method is carefully to pour or siphon off the solution.

To sum up we may conclude that the permanent preservation of most specimens for biological work is done in solutions whose preserving properties are due principally to their alcohol or formalin content. For extensive preservation in the field formalin is usually to be preferred because of the fact that a much smaller volume of fluid need be carried, since the usual dilution for formalin is 1 part formalin to 9 parts water, or in the case of the more delicate animals such concentrations as 1 part formalin to 20 or 30 parts water are used. It is not necessary to use distilled water to dilute the formalin, the writer usually finding it quite satisfactory in the field to use the nearest water source. For aquatic animals it is generally best to use water from the animals' habitat to accomplish the dilution. Thus for marine animals use the sea water, for fresh water animals use the water from the pond, lake, stream or spring in which the animal is found. In the writer's opinion this is usually preferable to the use of distilled water.

The ordinary concentration of alcohol is 70% or 7 parts alcohol and 3 parts water, and the dilution should usually be with distilled water. Formalin is not as satisfactory as alcohol for permanent preservation, however, for a formalin solution has a distinct tendency toward acidity, with the result that the specimens gradually decompose after a year or two. To preserve permanently such specimens they should be transferred to alcohol before the deterioration begins. Forms that possess calcareous shells, spicules or other structures of a similar nature, should if possible be placed directly in alcohol, since the acid formalin may seriously damage the specimens even within a short time. Alcohol of 70% concentration is preferable for permanent preservation of animals for the reason that it is sufficiently absorbed by the tissues after repeated changes to produce perfect preservation, while stronger concentrations are not only unnecessary but also may at times be harmful, because specimens that are too fully dehydrated in the preserving process are liable to become hard and brittle. Perhaps it should be pointed out that the effect of both formalin and alcohol is to a considerable extent to replace the water of the tissues with these substances, decomposition being prevented, since the tissue destroying bacteria are unable to grow except in the presence of a free supply of water. In removing the water, the preserving solution then prevents decomposition and at the same time stiffens the tissues so that the specimen does not collapse. As has been stated, 70% alcohol, if changed a sufficient number of times, will do both these things without injuring the specimen for either display or dissection purposes. Alcohol will fade the pigment colors from specimens more rapidly than will formalin, but these colors are fugitive in either case, the preserving medium which will permanently retain the color due to pigment in the skin of animals having not yet been discovered.

Special Preservatives

Including Anesthetizing, Killing and Fixing Reagents.

Many liquids have been tried as possible substitutes for alcohol, formaldehyde, and alcohol — formaldehyde combinations, but none has so far been found that can be considered entirely satisfactory as a general preservative. There are, however, a number of solutions that are of great value in the initial preservation of specimens for museums, for histological technique and for some other purposes. Some of these are suitable for use as permanent preservatives in which certain forms (not a great many) may be stored indefinitely. We list below the most generally used solutions, with brief comments on the value and limitations of each.

ACETIC ACID is a reagent which has the property of permeating tissues quickly. It furnishes a very efficacious means of rapidly killing contractile animals, but it has the disadvantage of softening them if they remain in it too long a time. Objects killed in acetic remain relatively transparent. In certain cases it is necessary to use a concentrated solution of the acid. Acetic acid is often mixed with chromic acid for killing and hardening noncontractile transparent animals.

ALCOHOL, ABSOLUTE. Absolute alcohol is valuable for the fixing of brains of dogs with rabies, and for other pathological nervous tissues.

ALCOHOL, ACIDIFIED. Acidified alcohol is occasionally used in the killing of some small marine forms. It may be made up in two ways:

Alcohol, 70 per cent	1 part
Chromic acid, 1 per cent	1 part

or

Alcohol, 50 per cent	100 c.c.
Hydrochloric acid (conc.)	5 c.c.

ALCOHOLIZED SEA WATER is used as an anesthetic in quieting small marine forms.

Sea water	100 c.c.
Absolute alcohol	5 c.c.

BOUIN'S FLUID is much used for preserving embryological and histological material intended for microscopic preparations.

Picric acid, saturated solution	75 c.c.
Formalin	20 c.c.
Acetic acid, glacial	5 c.c.

CARL'S SOLUTION is a formalin-acetic-alcohol mixture which finds a very wide use in the preservation of arthropods. It gives better internal preservation to insects than does any other fixative tried by the writer.

Alcohol, 95%	170 c.c.
Formalin	60 c.c.
Glacial acetic acid	20 c.c.
Water	280 c.c.

Omit the acetic acid until just before using.

CHLORAL HYDRATE is used in very weak solution, from 0.1 to 0.2 of 1 per cent made freshly in sea water, for narcotizing certain forms before fixing them. This method has the advantage that, if the animal after a certain time does not remain in the condition desired for preservation, it can be replaced in sea water, where it will regain power of motion and continue to live. Chloral is used for killing animals which live in the crevices of rocks, in incrustations of calcareous algae, and among colonies of serpulas and of madrepores, such being allowed to remain in the solution from six to twelve hours. It is not necessary to use chemically pure chloral hydrate.

CHLORETONE is useful in weak aqueous solutions for narcotizing many small aquatic forms.

CHROMIC ACID. Next to alcohol an aqueous solution of chromic acid is the most useful reagent, as it serves especially for killing and hardening gelatinous and soft animals. Objects should not remain in the fluid longer than is necessary, because they become to deeply tinged and are rendered brittle. Solutions are used in strengths of ½% to 1%, depending on the specimen being treated.

After treatment with the acid it is necessary to wash the animals with fresh water to avoid the formation of a precipitate when they are placed in alcohol, for if they are not well washed, they will in time acquire a greenish hue. Chromic acid is used mixed with osmic, acetic or picric acid, with corrosive sublimate ($HgCl_2$), and rarely with alcohol. The solutions are made in ordinary fresh water when possible, though occasionally salt water may be used. The solutions will not keep long in any case. That which has served once may be used again if it will not be too diluted when added to the water containing the animal and if too much time has not elapsed. When the solution has turned green after standing it is no longer fit to use.

CHROM-ACETIC MIXTURE No. 1:

Chromic acid 1 per cent	100 c.c.
Glacial acetic acid	5 c.c.

CHROM-ACETIC MIXTURE No. 2:

Chromic acid 1 per cent	100 c.c.
Glacial acetic acid	10 c.c.

CHROM-ACETIC-OSMIC. (See Flemming's solution.)

CHROM-OSMIC MIXTURE:

Chromic acid 1 per cent	100 c.c.
Osmic acid 1 per cent	2 c.c.

CHROM-PICRIC MIXTURE:

Chromic acid 1 per cent	50 c.c.
Kleinenberg's solution	50 c.c.

COCAINE. (Difficult to obtain because of narcotic laws.) A solution of cocaine is made by dissolving 2 grams of the hydrochloride in 100 c.c. of 50 per cent alcohol. This is a most excellent narcotizing medium, but its high cost prevents its extensive use. It is the best reagent thus far discovered for the treatment of gastropods. A few drops are carefully distributed over the surface of the water containing the animals, and the operation is repeated until the animals cease to respond to any stimulus. Other reagents that are used occasionally are chloroform, ether, and tincture of iodine.

COPPER SULPHATE. Solutions of from 5 per cent to 10 per cent strength, which are made with hot fresh water, are used alone or mixed with corrosive sublimate for killing larvae and delicate animals. The objects which have been treated with this reagent must be washed repeatedly with water or else they will not remain perfectly clear, owing to the formation of crystals within the tissues which render them opaque. If they afterwards prove to have been washed too little, the objects should be treated with several changes of an acid.

COPPER CORROSIVE SOLUTION:

Copper sulphate solution, 10%	100 c.c.
Corrosive sublimate, saturated aqueous solution	10 c.c.

CORROSIVE SUBLIMATE is much used as a fixing agent, because it has the property of permeating tissues rapidly and hardening greatly. It is used in concentrated solution in fresh or sea water, either cold or hot. In manipulations with sublimate, metallic implements must not be used because they decompose the solution and stain the preparations. The solution is made, when possible, with hot water for economy of time, and in vessels of glass or porcelain. All animals which have been prepared with this reagent can be used for histological research, but animals which have been treated with corrosive sublimate must be washed carefully and thoroughly in fresh water before they are placed in alcohol. Add a solution of iodine drop by drop until the alcohol remains permanently colored. This insures the entire removal of crytals of corrosive sublimate from the substance of the animal. If this precaution is not taken, the corrosive sublimate will be reduced and mercury will stain the animal black. Also, a black precipitate will form on the sides and bottom of the vessel. The amount of iodine to be used depends upon the size and character of the animal to be treated. Corrosive sublimate is most often used in mixtures as follows:

(1) CORROSIVE ACETIC SOLUTION

Corrosive sublimate, saturated aqueous solution	100 c.c.
Acetic acid, glacial	5 c.c.

(2) ZENKER'S SOLUTION

Potassium bichromate	2½ gms.
Sodium sulphate	1 gm.
Corrosive sublimate	5 gms.
Acetic acid	5 c.c.
Water	100 c.c.

(3) CORROSIVE CHROMIC SOLUTION

Corrosive sublimate, saturated solution	100 c.c.
Chromic acid of 1 per cent	50 c.c.

(4) HELLY'S FLUID

Potassium bichromate	2½ gms.
Sodium sulphate	1 gm.
Corrosive sublimate	5 gms.
Formalin	5 c.c.
Water	100 c.c.

FLEMMING'S SOLUTION is used for fixing small organisms and pieces of tissue for detailed microscopic structure. It must be washed out of the specimen in running water and the object preserved in alcohol.

Chromic acid, 1 per cent	25 c.c.
Osmic acid, 1 per cent	10 c.c.
Acetic acid (glacial)	5 c.c.
Distilled water	60 c.c.

FORMOL-ACETIC-ALCOHOL. This solution is an excellent one for general work since it acts as a good killing agent even for histological purposes, and has the additional advantage that the specimens may be allowed to remain in the solution for an indefinite time without injury. This solution is cheap, easily prepared, does not stain the hands or injure the clothing, and does not irritate the mucous membranes. It may be exposed to sunlight without deterioration, and has a tendency to bleach specimens immersed in it rather than to discolor them.

Formalin	6½ c.c.
Acetic acid, glacial	2½ c.c.
Alcohol, 50 per cent solution	100 c.c.

KAHLE'S SOLUTION. This modification of the formalin-acetic-alcohol mixture has proved to be an excellent preservative for insects. It penetrates well and insures good internal preservation.

Alcohol, 95 per cent	30 c.c.
Formalin	10 c.c.
Acetic acid, glacial	2 c.c.
Distilled water	60 c.c.

KLEINENBERG'S LIQUID is usually confined to the preparation of embryological subjects, with the single exception of Balanoglossus, which is killed with this solution for exhibition as well as for study.

Picric acid, saturated solution	100 c.c.
Sulphuric acid, concentrated	2 c.c.

Filter and add three times the volume of distilled water.

MÜLLER'S SOLUTION is a weak fixative in which tissues and small specimens may remain for a long time without injury.

Potassium bichromate	2 gms.
Sodium sulphate	1 gm.
Distilled water	100 c.c.

OSMIC ACID. In general, osmic acid is not used as much now as formerly because its use has several inconveniences. It hardens gelatinous forms well and preserves the transparency sufficiently, but its action is eventually too great, for the preparations become dark-colored and are rendered fragile; consequently they should remain in the solution only until they have acquired a light brown tint. Before they are transferred to alcohol they should be washed for some minutes in fresh distilled water, as should be done also with all animals which have been treated with any mixture containing even a trace of osmic acid. (See the chrom-osmic mixtures.)

PERENYI'S SOLUTION is recommended for yolk-containing material, which it does not render overly brittle.

Nitric acid, 10 per cent	40 c.c.
Chromic acid, 0.5 per cent	30 c.c.
Alcohol, 90 per cent	30 c.c.

POTASSIUM BICHROMATE is used as a 5 per cent solution for slowly hardening gelatinous animals without rendering them too fragile, when it is not possible to work with chromic acid. On account of the troublesome precipitate which forms when objects treated with bichromate are transferred to alcohol, the use of this reagent is not recommended. For bleaching the preparations before they are put into alcohol, use a few drops of sulphuric acid in water.

POTASSIUM BICHROMATE-OSMIC SOLUTION combines the hardening and preservative action of bichromate with the quick killing action of osmic acid.

Potassium bichromate, 5 per cent solution	100 c.c.
Osmic acid, 1 per cent solution.	2 c.c.

TELLYESNICKY'S FLUID is much used for amphibian and fish embryological stages.

Potassium bichromate	3 gms.
Acetic acid, glacial	5 c.c.
Distilled water	100 c.c.

As has been stated, preservation of animals for dissection purposes requires a preservative that will make certain that decomposition is completely checked without damage to the tissues, while preservation for museum purposes requires further that, where possible, the natural shape and color of the specimen be retained. To successfully preserve animals for dissection and display is a task worthy of the best efforts of the skilled preparateur and there are many problems yet to be solved before perfect specimens are to be had in laboratory and museum. We do not know of any general preservative that will preserve the natural colors of animal specimens. There is need of much experimentation in this field and we hope that eventually a really good form-and-color preservative may be discovered.

COMPARATIVE WEIGHTS AND MEASURES

Weights, Metric and Apothecaries'

1 grain = 0.065 gram (g)
1 ounce = 31.104 grams (g)
1 pound = 273.236 grams (g)
1 milligram (mg.) = 0.015 grains
1 centigram (cg.) = 0.154 grains
1 decigram (dg.) = 1.543 grains
1 gram (g) = 15.432 grains
100 grams (hg) = 1,543.235 grains or 3 ounces, 1 dram, and 43 grains
1,000 grams (kilo) = 15,432.349 grains, or 2 pounds, 8 ounces, 1 dram, and 12 grains

Weights, Metric and Avoirdupois

1 grain = 0.065 gram (g)
1 ounce = 28.35 grams (g)
1 pound = 453.59 grams (g)
1 gram (g) = 15.43 grains
100 grams (hg) = 3 ounces, 230 grains
1,000 grams (kilo) = 35 ounces, 120 grains

Liquid Measure, Metric and Apothecaries'

1 minim = 0.06 cc.
1 fluid ounce = 29.57 cc.
1 pint = 433.11 cc.
1 gallon = 3,784.88 cc.
1 cubic centimeter (cc) = 16.23 minims
100 cubic centimeters (dl) = 3.38 fluid ounces
1,000 cubic centimeters (liter) = 33.81 fluid ounces or 2.11 pints

Linear. Metric and English

1 meter = 39.37 inches
1 foot = 30.48 centimeters
1 inch = 2.54 centimeters
1 millimeter = .03937 of an inch

Thermometric

To reduce degrees Fahrenheit to degrees Centigrade, subtract 32° and multiply by 5/9; to reduce degrees Centigrade to degrees Fahrenheit, multiply by 9/5 and add 32°.

INDEX

Page

A

Abalone, collection 41
Acarina 32
Acetic acid 63
Acid, acetic 63
    chromic 64
    osmic 65
Acmaea, collection 41
Actinia, preservation 18
Actiniaria, preservation 17
Alcohol, absolute 63
    acidified 63
    biological 61
    denatured 61
    ethyl 61
    formol-acetic 65
    methyl 62
    rubbing 62
    wood 62
    procedure 62
Alcoholized sea water 63
Alcyonaria, preservation 16
Alligators; injection and preservation 53
Ameba, culture methods 9
Ammocoetes larvae 49
Amphibia 51
Amphibians, collection 51, 52
    injection and preservation 52
Amphioxus, collection and preservation 47
Amphipoda, preservation 31
Amphiporus, preservation 21
Anemones, collection 13
    preservation 17
Anesthetizing reagents 63
Annelida, collection 27
    preservation 28
Antedon, collection and preservation 39, 40
Anthozoa, preservation 16
Antipathes, preservation 17
Aplysia, preservation 45
Aquatic collecting 6
Arachnids, collection and preservation 32
Arachnoidea 31
Argas, collection and preservation 32
Argonauta 44
Arthropoda 30
Ascaris lumbricoides, collection 22
    megalocephala, collection 22
    preservation 23
Ascidiae, preservation 48
Asteroidea, collection 38
    preservation 39
Aurelia, collection 13
    preservation 16
Aves 53
Axinella 12

B

Balanoglossus, collection and preservation 47
Balanus, preservation 31
Barnacles, collection and preservation 30
Bdelloura, illustration 3

Page

Bdellouridae, collecting 19
Beroe forskalii, preservation 18
    ovata, preservation 18
Birds, collection 53, 54
    injection 55
    preservation 54
    study skins 54
Blood-suckers, see Leeches
Bonellia, preservation 24
Bopyrides, preservation 31
Botryllus, preservation 48
Bouin's fluid 63
Brachiopods, collection and preservation 25
Branchiostoma, collection and preservation 47
Branchipus, collection and preservation 30
Brittle-stars, collection 38
Bryozoa, collection and preservation 25
Bufo, collection and preservation 51, 52
Bugula, collection and preservation 25
Bullas, preservation 45

C

Cambarus, collection and preservation 30
Campanularidae, preservation 15
Carl's Solution 63
Cassiopeia, preservation 16
Cellepora, preservation 25
Centipedes, collection and preservation 32
Cephalopoda, preservation 45
Cercariae, collection and preservation 19
Cerebratulus, collection 20
    preservation 21
Cestoda, collection 19
    preservation 21
Cestus, preservation 18
Chaetognatha 24
Chaetopoda, collection 27
    preservation 28, 29
Charybdaea, preservation 15
Chevreulius, preservation 48
Chitons, collection and preservation 41
Chloral hydrate 63
Chloreton 63
Chordata 47
Chordates, collection 47
    preservation 47, 48
Chorioptes, collection and preservation 32
Chrom-acetic Mixture No. 1 64
    No. 2 64
Chrom-osmic mixture 64
Chrom-picric mixture 64
Chromic acid 64
Ciona, preservation 48
Circinalium, preservation 48
Cirripedia, preservation 31
Cladocera, preservation 31
Clam collecting, illustrations 43
Clams, collection 42
Clepsine, collection 28
Clonorchis 19
Cocaine 64

INDEX

Page
Coelenterata, collection 13
  preservation 14
Collecting, terrestrial 5
  aquatic 6
  general instructions 5
  marine 7
Comb-jellies, see Ctenophora
Conchoderma, preservation 31
Copepoda, collection and preservation 30, 31
  preservation 31
Copper corrosive solution 64
  sulphate 64
Corallium rubrum, preservation 17
Corals, collection 13
  preservation 18
Corrosive acetic solution 64
  chromic solution 64
  sublimate 64
Corydalus cornutus, preservation 35
Crabs, collection and preservation 31
Crayfish, collection and preservation 30
Crinoidea, collection 39
  preservation 40
Crisia, preservation 25
Criseis, preservation 45
Crustacea, collection 30
Crustaceans, preservation 31
Cryptobranchus, collection and preservation 51, 52
Ctenophora, collection and preservation 18
Cucumaria, preservation 40
Culture methods, protozoa 9
Cumacea, preservation 31
Cuttlefish, preservation 46
Cyanea, collection 13
Cyanide jars 34
Cyclostomata 49
Cyclostomes, collection and preservation 49
Cynthia, preservation 48

D

Daphnia, collection 30
Decapoda, collection and preservation 31
Demodex, collection and preservation 32
Denatured alcohol 61
Dentalium, collection 41
  preservation 41, 45
Dermacentor, collection and preservation 32
Diplodiscus, illustration 3
Dipylidium, collection 20
Dogfish, collection 49, 50
  injection and preservation 50
Dolichoglossus, collection and preservation 47
Dredge, illustration 33

E

Earthworms, collection 27, 28
  preservation 28, 29
Echinodermata 38
Echinoderms, collection 38
  preservation 39

Page
Echinoidea, collection 38
  preservation 39
Echinorhynchus, collection 22
Echiurus, preservation 28
Edwardsia, preservation 18
Elasmobranchii 49
Elasmobranchs, collection 49, 50
  preservation 50
Embalming 59, 60
Enteropneusta 47
Entomostraca, collection 30
Entoniscides, preservation 31
Ephyra, preservation 16
Ethyl alcohol 61
Euglena, collection and preservation 9, 10
  culture methods 10
Evadne, preservation 31

F

Fairy Shrimp (See Branchipus)
Fasciola hepatica, collection 19
  illustration 3
Fascioloides magna, illustration 3
Fiddler Crab 31
Fishes, collection of 50, 51
  preservation 51
Fissurella, collection 41
Fixing reagents 63
Flatworms, collection 19
  illustrations 3
Flemming's solution 64
Flukes, collection 19
  illustration 3
  preservation 21
Flustra foliacea, collection 25
Formol-acetic-alcohol 65
Formalin 61
Formalin neutral solution 62
  solution (standard) 62
Formanifera, collection 9
Fragarium, preservation 48
Frogs, collection and preservation 51, 52
Frog eggs, collection and preservation 51, 52

G

Gastropoda, preservation 45
Glossiphonia, collection 28
Gordiidae, collection 22
Gorgoderina, illustration 3
Gorgonia, preservation 17
Grantia, collection 12
  preservation 12
Gregarines, collection and preservation 11

H

Hairworms, collection 22
Haliotes, collection 41
Helly's Fluid 64
Hermit crabs 31
Hirudinea, collection 28
  preservation 29
Homarus, collection 30
  preservation 30
Horse ascaris 22

INDEX

Page
Holothuria, preservation 40
Holothurioidea, collection 38-40
  preservation 38, 39, 40
Hydra, collection 13
  preservation 14
Hydroidea, preservation 14
Hydroids, collection 13
Hydromedusae, preservation 15
Hydrozoa, preservation 14

I

Insecta 32
Insects, collection 34
  mounting 35
  preservation 35
Insect mounting equipment 35, 36, 37
  nets, illustrations 33
Isopoda, preservation 31

J

Jars, killing 34
Jellyfish, collection 13
  preservation 18

K

Kahle's solution 65
Killing reagents 63
Kleinenberg's Liquid 65

L

Lampreys, collection and preservation. 49
Lampsilis, collection 42
Leeches, collection 28
  preservation 29
Lepas, preservation 31
Lepidonatus, collection 27
Lima, preservation 45
Limpets, collection 41
Limulus planarian 19
  preservation 32
Lingula, preservation 25
Lobsters, collection 30
Loligo, see squids
Loligopsis, preservation 46
Lottia, collection 41
Loxosoma, preservation 25
Lumbricus, collection 27, 28
  preservation 28, 29
Lymnaea 19
Lymnaea, collection 41

M

Madreporaria, preservation 18
Mammalia 55
Mammals, collection 55
  injection 60
  preservation and
    embalming 59, 60
  skeletons 58
  study skins 55, 58
Man-o'-war, preservation 15
Margaropus, collection and
  preservation 32
Marine collecting 7
Measures, tables 66

Page
Medusae, preservation 15
Mermis 23
Metridium, collection 13
  preservation 17
Millipedes, collection 32
  preservation 32
Mites, collection and preservation 32
Molgula, collection and
  preservation 47, 48
Mollusca 41
Molluscoidea 25
Molluscs, collection 41
  preservation 44
Moniezia collection 20
Muller's solution 65
Mussels, collection and preservation 42-45
Mya, collection and preservation 42
Myriapoda 32
Mytilus, collection 42

N

Nautilus, collection 44
Necturus, collection and
  preservation 51, 52
Nemathelminthes, collection 22
  preservation 23
Nemertia, collection 20
  preservation 21
Nereis virens, collection 27
Nets, collecting 33
Neutral formalin 62
Notoedres, collection and preservation. 32
Nudibranchs, collection 41

O

Obelia, collection 13
  preservation 15
Octopi, collection 44
Octopods, preservation 45
Opalina, collection 10
Ophiopsila, preservation 39
Ophiura, collection 38
Ophiuroidea, collection 38
  preservation 39
Opisthobranchiata, preservation 45
Osmic acid 65
Ostracoda, preservation 31
Oysters, collection and preservation 42

P

Paguridae, preservation 31
Paramecium, collection 9
  culture methods 9, 10
Pectinatella, collection 25
  preservation 25
Pedicellina, preservation 25
Pelagia, preservation 16
Pelecypoda, preservation 44
Peltogaster, preservation 31
Pennaria, preservation 14
Perenyi's solution 65
Phascolosoma, preservation 24
Phoronidea, preservation 24
Phoronis, preservation 24
Phronima, preservation 31

INDEX

Page
Phyllosoma, preservation ... 31
Physa ... 19
Physa, collection ... 41
Physalia, preservation ... 15
Pisces ... 50
Placobdella, collection ... 28
Planaria, collection ... 19
  illustration ... 3
  preservation ... 20
Plankton towings ... 7, 30
  tow net ... 33
Planorbis ... 19
Platyhelminthes, collection ... 19
  illustration ... 3
  preservation ... 20, 21
Pleurocera, collection ... 41
Plumatella, collection ... 25
Pneumoneces, collection ... 19
  illustration ... 3
Podon, preservation ... 31
Polycyclus, preservation ... 48
Polycarpa, preservation ... 48
Polynoe, collection ... 27
Polyzoa, see Bryozoa
Potassium bichromate ... 65
Porifera, collection and preservation ... 12
Porpita, preservation ... 15
Portuguese Man-o'-war, preservation. 15
Protozoa, collection ... 9
  preservation ... 10
Preservatives ... 61
Psoroptes, collection and preservation. 32
Pteropoda, preservation ... 45
Pycnogonida, collection ... 31

R

Radiolaria, collection ... 9
  preservation ... 10
Rana, collection and preservation ... 51, 52
Reagents ... 63
Rediae, collection ... 19
Reptiles, collection ... 52
  injection and preservation. 52, 53
Reptilia ... 52
Rhipicephalus, collection and
  preservation ... 32
Rhizocephala, preservation ... 31
Rhizostoma, preservation ... 16
Rhodosoma, preservation ... 48
Rhopalea, preservation ... 48
Rotifers, collection ... 24
  preservation ... 24
Roundworms, collection ... 22
  preservation ... 23

S

Sacculina, preservation ... 31
Sagartia, preservation ... 18
Sagitta, preservation ... 24
Salpa, collection and preservation ... 47
Salpidae, preservation ... 48
Sand dollar, preservation ... 39
Sarcoptes, preservation ... 32
Scallops, collection ... 44
Scaphopoda, preservation ... 45

Page
Schistosoma ... 19
Schizopoda, preservation ... 31
Scyphistoma, preservation ... 16
Scyphozoa, preservation ... 15
Sea anemone, preservation ... 17
Sea-cucumber, collection ... 38
  preservation ... 38, 39, 40
Sea-pens, preservation ... 17
Sea-slugs, preservation ... 45
Sea-urchins, collection ... 38
  preservation ... [illegible]
Sharks, collection ... [illegible]
  injection and preservation ... 50
Shrimps, collection ... 30
Siphonophora, preservation ... 15
Sipunculoidea, preservation ... 24
Slugs, collection and preservation ... 41
Snails, collection ... 41
Snakes, injection and preservation ... 53
Sphinx larvae, preservation ... 35
Spiders, collection ... 32
  preservation ... 32
Sponges, collection ... 12
  preservation ... 12
Spongilla, collection ... 12
  preservation ... 12
Sporocysts, collection ... 19
Squalus, collection ... 49, 50
  injection and preservation ... 50
Squids, preservation ... 46
Starfish, collection ... 38
  preservation ... 39
Stichopus, preservation ... 40
Stomatopoda, preservation ... 31
Strobila, preservation ... 16
Styela, preservation ... 48
Stylatula, preservation ... 17
Suberites ... 12
Synapta, preservation ... 40

T

Taenia, collection ... 20
Tapeworm, collection ... 19, 20
  preservation ... 20, 21
Tellyesnicky's Fluid ... 65
Terebratulina, preservation ... 25
Teredo, collection and preservation .. 42, 44
Terrestrial collecting ... 5
Thyone, preservation ... 40
Ticks, collection ... 32
  preservation ... 32
Toads, collection and preservation ... 51, 52
Trachomedusae, collection ... 15
Trematoda, collection ... 19
Trichina, collection ... 22
Trochelminthes, collection and preservation ... 24
  preservation ... 24
Trombicula, collection and preservation. 32
Tubularia, preservation ... 14
Tubularidae, preservation ... 14
Tunicata, collection ... 47
  preservation ... 48
Turbellaria, collection ... 19
  illustrations ... 3

INDEX

Page
Turbellaria, preservation 19, 20
Turtles, collection and preservation..52, 53
Tyroglyphidae, collection and preservation 32

V

la, preservation 15
nia, preservation 46
brata 49
ra, collection 41
ella, preservation 11

Page

W

Weights, tables 66
Wheel animalcules 24
Worms, annelid 27
nermertine 20

Z

Zenker's solution 64
Zoantharia, preservation 17
Zoea, preservation 31
Zoobotrum, preservation 25

ANNOUNCEMENT

by the

GENERAL BIOLOGICAL SUPPLY HOUSE, Inc.

The *"Natural History of North American Animals with Special Reference to L Forms"* will be published as a series of booklets, each of which will cover one phylum of closely related phyla. This Natural History, when complete, will cover the anima from the Protozoa to the Mammals. The publication dates of each of the chapters in this Natural History will be announced later.

The present booklet, *"The Collection and Preservation of Animal Forms,"* is the f series of booklets included in the Natural History mentioned above. Due to the limit bilities for collecting both plants and animals, this booklet is necessarily incomplete a only the animals most commonly used and studied in biology laboratories. Other infor collection and preparation methods, not discussed here will, however, be given to tea write to our Service Department, explaining their special needs. In every case wher the desired information it will be passed on to other biologists without cost or obligat experience gained in over twenty years of collecting and preparing animals (as well fits us particularly well for recommending methods to teachers who wish to prepare part of their materials in their own laboratory.

The General Biological Supply House has, for many years, been a reliable sourc section and demonstration specimens of the finest quality. Our preparation laborator located in our own modern building and are supervised by university-trained biologists whom have had extensive teaching experience. The laboratories include divisions for aration of plant and animal specimens, slides, models, skeletons, life histories, equipment Turtox Products for Biology. The readers of this booklet who wish to purchase materials are invited to write to the General Biological Supply House for catalogs and desired information.

GENERAL BIOLOGICAL SUPPLY HOUSE, Inc.
761-763 East 69th Place
Chicago, Illinois :: U.S.A.

DATE OF ISSUE

This book must be returned within 3, 7, 14 days of its issue. A fine of ONE ANNA per day will be charged if the book is overdue.

This book is issued for

7 DAYS ONLY.